Incremental Growth Of Deciduous Tooth Enamel

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by

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I Wendy Birch confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

ABSTRACT

Deciduous tooth formation begins before birth and ends after birth. This makes it more difficult to construct a continuous developmental chronology for deciduous teeth than for permanent teeth. The discovery of the neonatal line in enamel and confirmation that it marks birth, allowed the expansion of deciduous dental chronologies, which until this time had been largely based on qualitative descriptions. The aim of this study was to use the daily incremental record in deciduous enamel to document rates of enamel formation and to use these data to produce regression equations that describe the average rates of deciduous enamel formation for each tooth type. These formulae can then be applied to all deciduous teeth even when daily increments are not visible, in order to estimate crown formation times and other events during crown development, as well as to determine the age at death where enamel formation has ceased prior to completion.

In permanent teeth, rates of enamel formation vary between 2.5µm per day at the EDJ to 6.5µm per day at the enamel surface. Seventy deciduous ground sections were examined and it was established that the daily rates in deciduous enamel varied less, with regional weighted means for all tooth types ranging from 2.85µm per day at the EDJ to 3.40µm per day at the enamel surface with extreme outliers of 2.07 to 4.97µm per day. The average daily incremental growth rate of enamel in deciduous teeth was calculated for each tooth type, the weighted mean of the apposition rate over both aspects (labial/buccal and lingual) and over all three regions (cervical, lateral and occlusal) for all tooth types was 3.23µm per day.

A key finding of this study was that there is a marked reduction in the enamel formation rate in the zone immediately following the neonatal line or following other accentuated striae assumed to be associated with stressful events. A catch-up phase usually followed these events, during which the previous rates recovered. These data provide clear evidence of enamel hypoplasia associated with both the birth process and other events that cause stress in perinatal life.

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CHAPTER 1: Introduction

The study of skeletal human remains can be used to construct a basic biological profile of the individual who owned the bones during life. Apart from helping to identify the individual, such profiles can be used to form demographic profiles of a population or derive conclusions about lifestyles, mortality rates and life expectancies. In an archaeological skeletal assemblage that contains juvenile remains, the age at death may be used to give an indication of the overall health and well-being of that particular population. While in a forensic investigation, the main objective is to establish a positive identification and ascertaining the age at death will assist in the discovery and confirmation of individual identity in order for legal investigations to proceed.

Teeth tend to be more resistant to the effects of inhumation and fire than bone, and as a result their study forms a large and significant part of the investigations of the osteologist, palaeontologist, physical anthropologist and forensic scientist. It is clear that the teeth are a vital component in any examination of skeletal material, not only because of their endurance but also because of their ability to record the biology of the body during the developmental life of the individual. The dentition begins its formation very early in gestation and it does not complete its development until the third decade of life. The insults that the body and the dentition have received during this time may be recorded in the skeleton, however while bone remodels during life thus obliterating any earlier changes, these changes are often permanently recorded in the teeth. The aim of this thesis is to investigate the incremental growth of deciduous enamel, paying particular attention to one incremental structure, the neonatal line. The neonatal line is an example of one of the insults mentioned above that the dentition is subjected to and which, as its name suggests occurs during the birth and subsequent neonatal period of the developing infant.

The discovery of the neonatal line and proof of its neonatal origin allowed the development of deciduous dental chronologies, which until this time had been largely based on descriptive text. The development of these chronologies and

the addition of a new chronology resulting from the production of regression formulae based on the daily incremental growth of enamel, form the core of this work. The main aim of this work is to define more clearly the start and finish of enamel matrix secretion in the deciduous crown, in order to improve methods for estimating the age at death of juvenile human remains from forensic, archaeological and palaeontological contexts.

The second chapter presents a general background to the anatomy of a typical tooth and the deciduous dentition. Chapter 3 describes the development of the deciduous dental chronologies pertaining to crown formation times and discusses the limiting factors, in particular those experienced with the material and the methods used to obtain such data. The incremental nature of the neonatal line is then discussed, followed by a description of the pre- and postnatal enamel in Chapter 4. Chapters 5 and 6 present the experimental aspect of this work and describe the methods used to obtain the data from which the resultant regression formulae were produced. These formulae are then used to present a new chronology for deciduous crown formation. Chapter 7 describes three case studies using the formulae that were developed in the experimental section and compares the results obtained to the medical histories of each individual. Chapter 8 presents the conclusions and a discussion of the experimental work, along with suggestions for further work in this area. Finally Chapter 9 includes publications related to this study which have been included for additional information.

CHAPTER 2: The Human Deciduous Dentition

2.1 Introduction

The aim of this second chapter is to present a general background regarding deciduous teeth, as well as to describe some of the structures that are referred to throughout this thesis. As discussed in greater detail below, teeth consist of two main components, the crown and the root(s) and they are formed from three mineralised tissues, the enamel, dentine and cementum, which surround an inner core of loose connective tissue, known as the dental pulp. As the underlying principle of this thesis is to investigate the incremental nature and structure of enamel in the deciduous dentition, the main focus of this chapter and indeed this thesis, concentrates on the crown and the enamel; the root, the other two mineralised tissues and the permanent (secondary) dentition will not be discussed in detail.

This chapter commences with a general description of the anatomy of a typical tooth and of the composition of enamel which covers the crown. The deciduous dentition is then discussed.

The information covered in this chapter has been collated from several reference sources, from which a more detailed description of dental anatomy and crown morphology can be obtained, (Avery 1994; Bath-Balogh and Fehrenbach 2006; Berkovitz et al. 1992; Berkovitz et al. 2005; Bhaskar 1991; Brand and Isselhard 1994; Hillson 1996; Schroeder 1991; Ten Cate 1998; van Beek 1983).

2.2 Dental Anatomy

2.2.1 General Description of a Typical Tooth

Teeth play an important role in many functions of the human body. They constitute the part of the skeleton that directly interfaces with the environment, they are essential for acquiring and processing food which is subsequently passed farther along the digestive tract and they also protect the oral cavity. They are necessary for proper speech and their appearance can be of positive sexual attraction (or not). As mentioned in **Chapter 1**, to the osteologist, physical anthropologist and palaeontologist, teeth are probably the most important elements of the skeleton as they can provide a huge amount of detailed information about the individual possessing them. Teeth can provide information about biological age, sex, health, diet and even the evolutionary position of extant and extinct mammals, hominids included.

Each tooth is divided up into two parts, the anatomical crown and the anatomical root(s), these merge together at the slightly constricted cervix of the tooth (see **Figure 2.1**). The crown is the part of the tooth that projects into the oral cavity and provides the biting surface, the root is the part that is embedded into the bony alveolar socket of the jaw.

The crown of the tooth consists of a layer of hard, inert, non-vital and acellular enamel which is supported by the slightly less mineralised, more resilient and vital connective tissue, dentine, which itself surrounds the pulp chamber enclosing the dental pulp. The main bulk of the whole tooth consists of dentine, the crown portion is covered with enamel and the root portion is covered with a thin layer of a bonelike tissue called cementum. The enamel of the crown and the underlying dentine join at the enamel-dentine junction (EDJ), while the enamel and the cementum of the root join at the enamel-cementum junction; the line that demarcates the union of these two junctions is known as the cervical line and it occurs at the cervix of the tooth.

In mammals the tissues that support the teeth in the jaws, known collectively as the periodontium, include the alveolar bone forming the root sockets, the periodontal ligament which is a fibrous connective tissue that attaches the cementum of the root to the alveolar bone and which provides an attachment with enough flexibility to withstand the massive forces of mastication and the gingivae, which is the component of the oral mucosa that covers the alveolar bone and forms a collar around the cervix of the tooth. Teeth in the upper jaw are termed maxillary teeth as they are anchored into the alveolar bone of the maxilla, while teeth in the lower jaw are termed mandibular teeth as they are anchored into the alveolar bone of the mandible.

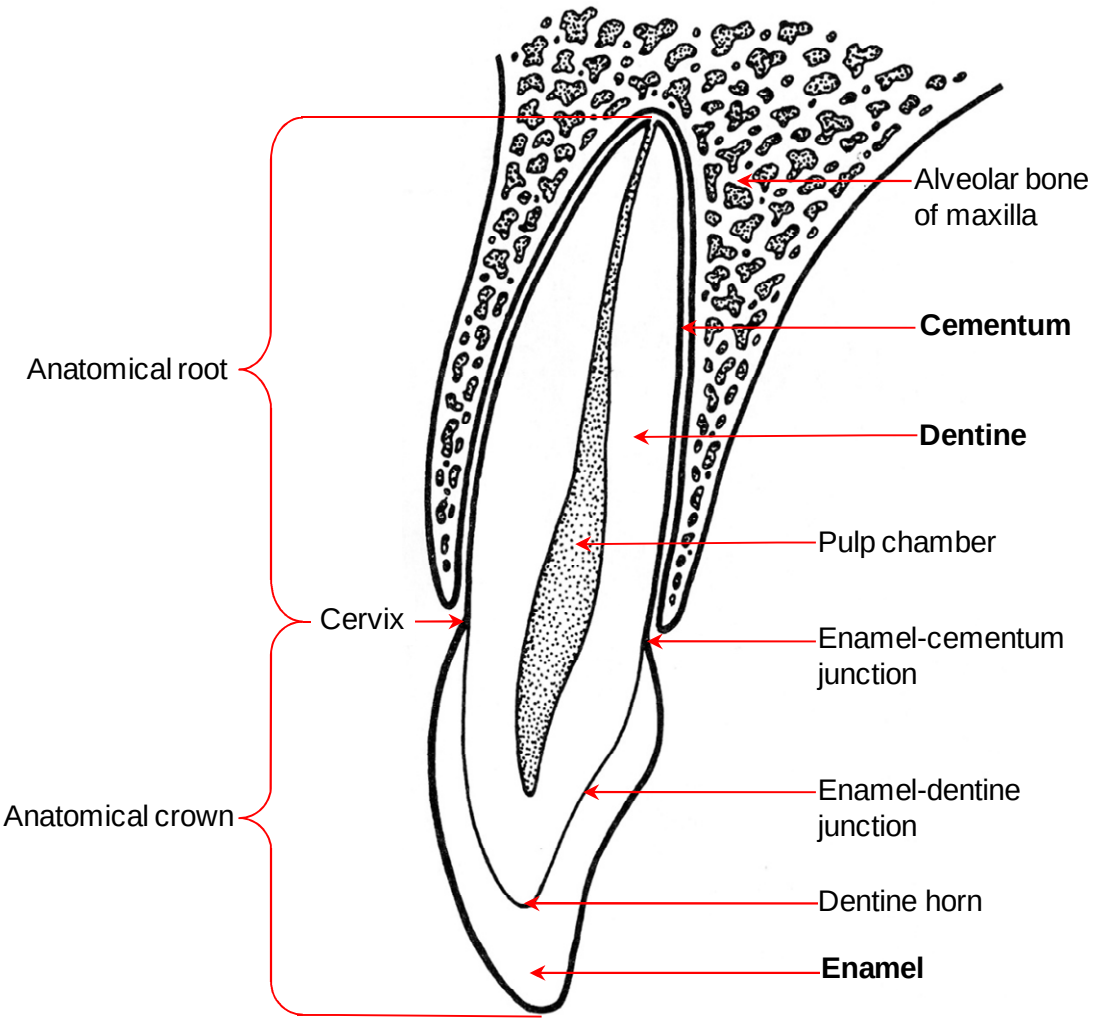


Figure 2.1: Longitudinal cross-section through a typical anterior maxillary tooth in situ and its surrounding alveolar support, this longitudinal section reveals the three mineralised tissues that form the tooth. Adapted from Brand and Isselhard (1994:241).

2.2.2 Enamel Thickness and Composition

Mature enamel is the hardest and most highly mineralised substance produced by the human body and together with dentine it makes up the dental crown, (see **Section 2.2.1**). In a healthy dentition the enamel is the only portion of the tooth that is usually seen clinically, because it covers the entire anatomical crown and completely coats the underlying supporting dentine. Enamel varies in thickness over the surface of the crown being thickest over the cusps and incisal edges it then decreases gradually to become a very thin layer at the cervical margin. Although the thickness of enamel is more consistent in deciduous teeth than it is in permanent teeth, it is much thinner. The average enamel thickness is 0.5-1.00mm (van Beek 1983:11) increasing up to 1.3mm over the unworn cusps (Berkovitz et al. 2005:14 and 102), compared to the permanent crowns where the average thickness over the unworn cusps is approximately 2.5mm (Berkovitz et al. 2005:14 and 102).

Enamel provides a hard surface for mastication and speech and it also provides the whiteness of a healthy smile. Enamel on its own can be various shades of bluish white, which is seen on the translucent tips of newly erupted incisors, but it appears as various shades of yellow-white elsewhere because of the underlying dentine. Since enamel is translucent, the colour of the underlying dentine strongly affects the appearance of a tooth, the thicker the enamel, the whiter it appears. Not only does the colour of enamel vary with its thickness, it also varies with its degree of mineralisation. The more mineralised the enamel is, the more it lends itself to translucency. The enamel on deciduous teeth is more opaque than permanent enamel and this gives the crown a bluish-white appearance (Bath-Balogh and Fehrenbach 2006).

Mature enamel is totally acellular, avascular and has no nerve supply within it. It consists of an inorganic mineral component of which the principal mineral is calcium hydroxyapatite, comprising of about 88-90% of the tissue by volume, which corresponds to about 95-96% by weight (Berkovitz et al. 2005:102). The mineral content of enamel increases from the enamel-dentine junction to the surface (Wilson and Beynon 1989). The remaining components of mature

enamel consist of water (about 5-10% by volume, corresponding to 2% by weight of enamel) and a small amount of fibrous organic material (about 1-2% by weight) (Berkovitz et al. 2005:104). Although proteins, peptides and free amino acids may account for less than 1% of the weight of mature enamel, in immature enamel this amount is about 25-30% as the developing enamel matrix is almost entirely proteinaceous (Berkovitz et al. 2005:312), most of this protein is subsequently removed during the maturation stage of amelogenesis.

The properties of enamel vary at different regions within the tissue. Surface enamel is harder, denser, less porous and less soluble than sub-surface enamel. Hardness and density decrease from the surface towards the interior, and also from the incisal/cuspal tip towards the cervical margin (Berkovitz et al. 2005; Wilson and Beynon 1989). The exceptionally high mineral content and dense mineralisation of mature enamel accounts not only for its hardness and strength but also for its brittleness. Although enamel has a low tensile strength it is so brittle that it cannot withstand the forces of mastication without fracture unless it has the support of a more resilient tissue such as dentine, which helps to compensate for its brittleness. Enamel ranks 5-8 on the Mohs hardness scale (Schroeder 1991:79), while dentine which is less mineralised and less brittle ranks 3-4 in hardness. Although it is brittle, enamel withstands both shearing and impact forces well. Its abrasion resistance is also high, allowing it to wear down very slowly, which is an important property as enamel cannot undergo repair or replacement. This inability to repair itself is caused by the cells responsible for the formation of enamel, the ameloblasts, these cells cover the entire surface of the enamel as it forms but they are lost as the tooth emerges into the oral cavity. The loss of these cells renders enamel a nonvital and insensitive tissue, so when it is damaged or destroyed (usually by abrasion[1], attritional[2] wear or caries), it cannot be replaced or regenerated. However, although enamel is a dead tissue, in the strict biological sense, it is not a static tissue as it can undergo mineralisation changes; it is permeable and ionic exchange can occur between the enamel and the environment of the oral cavity particularly with the saliva.

[1] Mechanical loss of dental tissue resulting from frictional wear, for example from excessive tooth brushing.
[2] Mechanical loss of dental tissue resulting from tooth-to-tooth contact by mastication or parafunctional habits.

In addition to being durable, the densely mineralised enamel surface is smooth. This smoothness gives the crown a certain self-cleaning ability, making it difficult for food particles, bacteria, sticky carbohydrate material and other debris to adhere to the surface of the tooth crown. This self-cleaning ability of enamel and its extreme hardness and resistance to wear make it a nearly perfect outer covering for the crown.

Enamel is composed of prisms which run from the enamel-dentine junction to the surface of the tooth. These prisms are the product of appositional secretion by the ameloblasts, that is to say, the ameloblasts secrete enamel in layers one on top of another. This type of growth results in the formation of concentric layers that are delineated by ‘growth’ or ‘incremental lines’, as a result these layers are therefore characterised by the regular and rhythmic manner in which enamel formation occurs. One form of incremental line results from the daily physiological rhythm in cellular activity; these increments are commonly referred to as enamel cross-striations. Daily cross-striations can be used to establish the rate of enamel formation and it is these incremental structures that are exploited in this thesis to develop regression formulae in order to establish crown formation times for the deciduous dentition.

Another form of incremental line is the result of a longer period rhythm, these incremental lines are spaced several days apart and it is this rhythm which underlies the striae of Retzius in permanent enamel; although these striae are much less prominent in deciduous enamel.

During formation and mineralisation, enamel and dentine are extremely sensitive to variations in metabolic processes, so much so that alterations in the internal environment of the body are often recorded as accentuated striae in the incremental layer that was developing at the time. The neonatal line in enamel has been reported as being an accentuated stria of Retzius (Andresen line in dentine), produced as the result of a disturbance of enamel formation and mineralisation and which occurs at the time of birth and during the immediate neonatal period.

2.2.3 General Description of the Deciduous Dentition

Humans, like all primates are diphyodont that is, they possess a primary dentition that is replaced by a secondary dentition. It is the first, primary or deciduous dentition that this thesis is concerned with. The primary dentition consists of twenty teeth, often referred to colloquially as ‘milk teeth’, ‘baby teeth’ or ‘temporary teeth’. The reason humans have two dentitions is to accommodate the growth of the face and jaws. Children have small faces with small jaws and consequently these small jaws can only carry a few small teeth. With growth, an increase in the size of the jaws occurs, necessitating not only more teeth but also larger teeth; as teeth cannot increase in size after they are fully formed, the deciduous dentition becomes inadequate and so these teeth are exfoliated (shed) and replaced by those of the permanent dentition. Normally eruption of the deciduous dentition commences at approximately six months and completes at about 2.5 years. The first permanent tooth erupts, usually at the age of six years. Deciduous teeth are extremely important for the proper formation of the bones of the maxilla and mandible, development of the muscles of mastication, as well as for the eventual location, alignment and occlusion of the permanent teeth.

Deciduous teeth generally differ from the permanent teeth, as they are usually much smaller and lower than their permanent successors and overall they exhibit relatively constant forms, that is, they display much less morphological variation and exhibit only limited individual variations unlike the permanent teeth (Hillson 1996; van Beek 1983). Deciduous crowns are more bulbous in shape, than their permanent successors; the narrow constricted cervix and the pronounced cervical enamel margin, which tends to form prominent bulging ridges instead of terminating smoothly at the enamel-cementum junction, further accentuates this shape. The enamel margin also tends to extend around the tooth in the same horizontal plane and has less of a sinuous path when compared to that of the permanent crowns. When newly erupted and unworn, deciduous cusps are also usually more pointed than those of the corresponding permanent teeth.

CHAPTER 3: Estimation of Age and the Timing and Sequence of Deciduous Crown Formation

3.1 Introduction

The state of dental development provides the best evidence for age estimation at death of juvenile human remains (Scheuer and Black 2004), whether they are complete or fragmentary or are from an archaeological or forensic context. As the determination of crown formation times can be used as a basis of this estimation and as part of the histological section of this thesis involves the calculation of the time it takes for deciduous crowns to form, a discussion of the previous research that has been conducted in this area has been included. The following chapter attempts to present the convoluted evolution of the chronology of the deciduous crown, detailing crown initiation and completion times and the methods that have been used to establish these chronologies.

This chapter starts with an overview of age and the terminology used to discuss it. The methods that have been used to produce these chronologies and the advantages and disadvantages of each are then discussed. The historical review is then presented, culminating in the presentation of five summary tables detailing this work. The final section of this chapter discusses the limiting factors in the creation of such a chronology.

3.1.1 Age

Chronological age (postnatal age) is the actual age of an individual; it is normally calculated from the day of birth. In general, chronological age is positively correlated with growth and development and so estimates of the age of an individual often utilize the many incremental changes that occur during development. However, the relationship between growth, development and chronological age is not linear and therefore the concept of ‘biological age’ is used to indicate how far along the developmental continuum an individual has

progressed; biological age identifies the developmental changes that occur in living tissues.

Age estimation of an individual involves first establishing a biological age and then attempting to correlate this with a chronological age. Biological age may be expressed as either 'skeletal age' or 'dental age' and it is generally recognised that '*the relationship between chronological age and dental age is stronger than that for chronological age and skeletal age*', (Scheuer and Black 2004:3). The dental age of an individual can be estimated by examining the extent of dental eruption or the state of formation or maturation of the developing tooth germ; of the two, '*formation of teeth appears to be more robust to environmental influences*' (Smith 1991:143). It is the state of formation and in particular the initial mineralisation of the enamel crown that this thesis is concerned with. However, in order to estimate the dental age of an individual or isolated tooth, the specimen in question must be compared to a 'known standard', unfortunately by doing this, incompatibilities are inevitably introduced. For this reason, even though the establishment of age at death of juvenile remains can be considered more accurate than establishing the age of death of adult remains (Scheuer and Black 2004; Smith 1991), due to the decreased time span of human growth relative to the total life span over which age variability is assessed, the aging of juvenile remains based on dental development is nevertheless always only an '*estimation*' (Scheuer and Black 2004:3).

A vast amount of research has been carried out in order to attempt to establish 'known standards' that can be used to help estimate dental age by observing the state of dental development, most of this research however, involves the state and degree of dental eruption. There is a limited amount of research regarding the development of the crown and limited attempts have been made to establish a chronology of deciduous crown formation. Different techniques and methods have been used by different authors using material from a variety of sources to try to establish this chronology and unfortunately this has lead to a degree of confusion in the reported literature due to several main variables. The specimens used historically to study prenatal development of the deciduous crown, are by their nature, from an entirely different source of material than the

specimens used to study postnatal crown development and this immediately introduces a potential source of error as the material used is from different individuals. In addition to this, the identification of initial mineralisation depends on the technique used to observe it, as well as the existence of inconsistencies and inaccuracies incurred in the determination of the actual age of the specimens examined. Many researchers have also used different terms and measurements to describe the developmental phases of the material studied, while some of these terms and measurements are established clinical definitions, others are not universally accepted. Their usage also varies in different contexts and in different countries; in order to clarify this, the terms used in this thesis are defined below. Not surprisingly, each of these variables contributes to the non conformity of the reported developmental sequences and crown formation times.

3.1.2 Terminology Used In Previous Research

During the prenatal period, ‘chronological age’ does not technically exist, as it is rarely possible to establish a definite starting point (i.e. fertilization) with any certainty. The exact date of insemination is rarely known and tends to be restricted to cases of rape or assisted fertilization (Blackmon et al. 2004; DiPietro and Allen 1991). Clinicians and embryologists record age slightly differently, which has led to confusion in the literature (Blackmon et al. 2004; O'Rahilly 1997; O'Rahilly and Muller 2000). In a clinical context, the only known date is usually that of the first day of the last normal menstrual period (LMP) of the mother, but even the accuracy of this date may be affected by factors such as post-fertilization bleeding, inconsistencies of maternal recollection or intentional falsification. In addition, as the interval between LMP and conception is unknown and slightly variable, attempts to define a ‘true’ clinical age imply a precision which is simply not possible. Clinically, normal term is calculated as 280 days (40 weeks / 10 lunar months).

Unlike clinicians, embryologists calculate age from the time of fertilization which takes place approximately two weeks after the first day of the last normal

menstrual period, the anatomical prenatal age averages 266 days (38 weeks / 9.5 lunar months). However, this can vary with the interval between ovulation and fertilization.

So because of the variations in menstruation, ovulation, insemination and fertilization mentioned above, it is extremely rare to actually know the precise age of an embryo or fetus (Bagnall et al. 1975; Birkbeck 1976; Blackmon et al. 2004; DiPietro and Allen 1991; Kellokumpu-Lehtinen 1984; Patten and Philpott 1921; Roberts 1976; Tucker and O'Rahilly 1972; Wigglesworth 1996a).

The prenatal timescale used throughout this thesis is defined in **Table 3.1** below.

Table 3.1: Time scale of the entire prenatal period.

Days	Weeks Post Fertilization	Months
1-28	1-4	1
29-56	5-8	2
57-84	9-12	3
85-112	13-16	4
113-140	17-20	5
141-168	21-24	6
169-196	25-28	7
197-224	29-32	8
225-252	33-36	9
253-266	37-38	9.5

Adapted from Scheuer and Black (2004:5).

Throughout this thesis birth has been taken as occurring after 273 days (39 weeks / 9.75 lunar months), as this appears to be the most recent figure available for the duration of pregnancy until the occurrence of spontaneous birth (Davidoff et al. 2006).

In their fetal aging research, Croft et al. (1999) refer to the term 'gestational age' however, they may have actually meant 'menstrual age', this confusion introduces a possible error of up to two weeks depending on which definition of 'gestational age' is used. As mentioned above to a clinician the term 'gestational age' is measured from the first day of the LMP, while to an embryologist 'gestational age' is measured from the day of fertilization. This confusion is a common finding throughout developmental research (Blackmon et al. 2004; O'Rahilly and Muller 2000) and Croft et al. (1999) is only one example of the confusion caused by the lack of firmly defined terminology. The terms used throughout this thesis are defined in **Table 3.2** below.

Table 3.2: Terms commonly accepted by clinicians, embryologists and skeletal biologists that are used throughout this thesis. Where different definitions have been used by other authors these have been clarified at the time.

Embryo	First 8 weeks of intrauterine life
Fetus	From 8 weeks intrauterine life to birth
Trimester	A third of the time of normal pregnancy, thus 1st trimester = 1-3 months, 2nd trimester = 3-6 months, 3rd trimester = 6-9.5 months
Preterm	From <37 weeks (258 days) LMP
Full-term	From 37-42 weeks (259-293 days) LMP
Post-term	>42 weeks (294 days) LMP
Stillbirth	Infant born dead after gestational period of 28 weeks
Perinate	Around the time of birth
Neonate	First 4 weeks after birth
Infant	Birth to the end of the first year
Early childhood	To the end of the 5th year, often pre-school period
Late childhood	About 6 years to puberty

Adapted from Scheuer and Black (2004:4 and 6).

Not only are there current differences in the terms and methods used to determine the age of the fetal material studied, but historically, age was expressed in terms of the crown-rump length, crown-heel length or foot length of the fetus, or any combination of these. These measurements were then converted to a fetal age using other researchers conversion data (Birkbeck 1976; Noback 1922; Scammon and Calkins 1923; Scammon and Calkins 1929; Streeter 1920; Wigglesworth 1996a) therefore introducing more potential sources of error. Ultimately, however, these conversion data have been derived

from populations in which the researcher has accepted the accuracy of the mother's recollection of her dates of LMP, (Wigglesworth 1996a) unfortunately these dates are not always reliable (DiPietro and Allen 1991; Kellokumpu-Lehtinen 1984).

3.2 Material Used In Previous Research

In general, early dental development has been studied using aborted embryos and fetuses. In contrast, much postnatal information comes from radiographs of living children, although there are a few radiological and histological studies on post-mortem remains. There is also a wealth of archaeological data from the skeletons of individuals whose age at death has been estimated from morphological criteria or obtained via documentation. However, because of this variety of source material and the methods of observation used to examine it, it is vital that in any study of individuals of unknown age that if at all possible, the provenance of the material used for comparison is identified and where appropriate, comparable. Unfortunately this has not always been the case in the published research and this has often resulted in confusion.

3.3 Methods Used To Investigate Deciduous Crown Chronologies

The initiation of mineralisation has been described as commencing with the '*formation of a tiny increment of dentin*' at the dentine cusp tip (Schour and Massler 1940a:1921) and the completion of crown formation has been described as occurring when '*enamel formation ceases*' (Schour and Massler 1940a:1921). However determining when this has actually occurred can be extremely problematic. In addition, different methods have been used to study the formation and development of the deciduous dentition, which has complicated matters even further. Of the varied methods used to examine initial crown formation, each technique has its own advantages and disadvantages, depending on the information that is required. The advantages and limitations of each of these methods will now be discussed.

3.3.1 Radiography

Only one form of investigation can really be used to observe crown formation of the deciduous dentition in living infants and this is radiography. The best method to utilise radiological imaging is to observe the developing teeth and jaws at regular intervals and then to carry out a comparative study of the findings in identical areas of the same individual at different ages. This allows the developmental changes that occur to be visualised over time in the same individual.

One limitation of radiography pointed out by Logan and Kronfeld (1933:388) is that in the first months after birth, good radiographs are '*extremely difficult to obtain owing to lack of cooperation by the patient*'. In addition to this, only living infants who are usually ill have cranial radiographs taken and these images may not be suitable for dental developmental research, there is also the possibility that the resultant radiographs may not be a true representation of normal dental development due to the pathological requirement of the radiograph. Furthermore, few ethics committees today will allow an infant sample to be exposed 'unnecessarily' to radiation at repeated intervals throughout their childhood, although with the advent of CT and MRI imaging this type of longitudinal study may be possible in the future.

Several researchers have used radiography on deceased infants or anatomical specimens, (Boller 1964; Gantz 1922; Hess et al. 1932; Logan and Kronfeld 1933; Turner 1963), which has the advantage of full patient cooperation and allows for a variety of imaging positions, increased exposure times and allows multiple attempts to produce the most suitable images. However, it does mean that a longitudinal study is impossible as the use of radiography on deceased infants only permits a cross-sectional study, illustrating only one snap-shot in time of dental development.

McCall and Wald (1940:97) defended the use of radiographs to study dental development and stated that radiography provides a '*convenient, quick and inexpensive way to make comparative studies of the development of the teeth and jaws in the fetus and the child*'.

3.3.2 Study of Human Skulls

Another method used for examining tooth germs, crown formation and establishing the degree of mineralisation that has occurred at different ages is by the examination of dried juvenile skulls. Using this method it is necessary to remove the outer bony plates of the maxilla and mandible in order to gain access to the developing germs. Examples of these aged dried specimens are often found in dental histology textbooks, for examples see Ten Cate (1998:290) Brophy (1916a:806) and Broomell and Fischelis (1913:421). Some text books contain complete series of dried skulls illustrating the development of teeth at various ages (Mummery 1924:412-15; Noyes 1921:347-362; Noyes et al. 1938:Plate XII and XIV).

Unfortunately, in dried skulls the soft tissue which usually supports the developing crowns is absent and this invariably results in the displacement or loss of the very friable developing crowns. Another disadvantage of using skeletal specimens is that although these specimens may clearly illustrate the degree of crown formation in older juveniles, because the delicate structures of the developing germs undergo decomposition, leaving behind only those germs which were fairly well formed and mineralised at the time of death, this method is therefore not entirely suitable for the study of the very early stages of deciduous crown formation.

Again a longitudinal study is impossible as the use of fetal skulls to observe crown formation only permits one snap-shot in time of deciduous dental development.

3.3.3 Dissection

While the above method utilises dried skeletal remains other researchers have attempted to illustrate crown formation in young juveniles by soft tissue dissection (Boller 1964; Broomell and Fischelis 1913; Nomata 1964). This involves dissecting the tooth follicles from their crypts, together with the overlying mucous membrane and periosteum. Then by further dissecting the delicate mineralised caps from the underlying pulp tissue it is possible to follow the progress of mineralisation at different ages. One of the earliest and most detailed dissection studies of human tooth germs was carried out in 1913 by Broomell and Fischelis. From their dissections Broomell and Fischelis (1913:418) were able to report that at '*about the fourth fetal month preparations for the calcification of the deciduous teeth are begun*'. This dissection method allowed Broomell and Fischelis to report the initial mineralisation of deciduous crowns at a much earlier stage of development than had previously been seen in radiographs or by the examination of dried skulls (Logan and Kronfeld 1933). Hess et al. (1932), emphasized the point that radiographs and anatomical dissections do not produce similar results when observing crown formation, they stressed that mineralisation always appears much further advanced in anatomical dissections than it does in radiographs of the same specimen. They stated that the reason why mineralised areas which are visible to the naked eye when a tooth germ is exposed in the jaw are not visible on a radiograph is because during the very early stages of crown development the pulp is covered with an '*inorganic cap too thin to project a radiographic shadow*' (1932:1054). In 1935b Kronfeld reported that this was not in fact the case and that from his investigations of the initial mineralisation of the first permanent molar, using jaw specimens from which all of the investing soft tissue had been removed, he found that whenever initial mineralisation was found histologically, a corresponding shadow could be found on the radiograph. Although, he then stated '*it was sometimes necessary to use a magnifying glass or to enlarge the film before it could be clearly seen'* (Kronfeld 1935b:1138). However, in his work in 1935c Kronfeld stated that the appearance of initial mineralisation was identifiable two to six months earlier in serial histology sections than it was in radiographs. In 1939 Kronfeld and Schour suggested that initial mineralisation was visible in histological sections two months in advance of that first seen by

radiography; a year later in 1940 Schour and Massler confirmed this two months time difference. As a radiograph is a record of density it does not record the apposition of the un-mineralised matrix or the early radiolucent stages of mineralisation, as a result of this, mineralisation times from radiographs are usually shorter than the actual times taken for either initial mineralisation or full crown completion.

Unfortunately, during the very earliest stages of enamel formation the enamel cap is extremely delicate and often difficult to demonstrate, Logan (1935:4) suggested that this is because '*at birth, or at the age of a few months, most structures attached to the maxilla or mandible are not sufficiently differentiated to be good subjects for anatomic dissection*'. So like the use of dried skeletal specimens, dissection cannot adequately illustrate the very early stages of dental development.

Again a longitudinal study is impossible as the use of fetal specimens to observe crown formation only permits one snap-shot in time of deciduous dental development.

3.3.3.a Dissection – Alizarin Staining

Although the use of fetal specimens cleared with potassium hydroxide and cedar oil was used in 1922 by Gantz to observe developing teeth in situ, it was not until the work of Kraus (1959a and 1959b) and Kraus and Jordan (1965) that alizarin red S stain was used to investigate initial mineralisation times in large samples.

This technique utilises simultaneous clearing and staining of the mineralised tissue with potassium hydroxide and alizarin red S. In alizarin stained tissues all of the mineralised areas are stained red, whilst the un-mineralised areas remain clear, which is why this is such an ideal technique for distinguishing between mineralised and non mineralised tissues.

Kraus (1959a and 1959b) first cleared and stained the jaws of his specimens and then dissected the developing tooth buds from their bony crypts. While Kraus and Jordan (1965) first dissected the tooth follicles intact from their crypts, stained them and then under low magnification carefully dissected the embryonic crown from the rest of the follicle.

Kraus (1959b:1130) preferred the use of alizarin to radiography, as radiography '*does not detect the earliest formations of calcified material*'. Kraus and Jordan (1965:29) also added that '*neither histological sections nor radiographs of human fetal dentitions can provide accurate or complete information about dental morphogenesis*' as the soft parts of the crown are difficult to distinguish and the view radiographs provide is only in two-dimensions. Kraus and Jordan (1965:29) stated that even though reconstructions of the embryonic crown can be produced from serial sections '*the process is time consuming and costly and yields at best a distorted picture*'. They concluded that for morphological studies '*there is no substitute for examining the total intact bud or crown*' and that the dissection and alizarin staining method that they used is '*simple, direct and permits unlimited sample size*' (Kraus and Jordan 1965:29).

3.3.4 Histology

Unlike dissection, the use of histological sections allows crown formation, as well as the development of the jaws and associated oral and facial structures to be examined without any distortion, change in topography or tissue loss, this makes it possible to study even the earliest and most minute developmental changes in situ. Two types of histological section can be used to study dental structures these are the demineralised section and the ground section. Kronfeld (1937:174) stated that demineralised and ground sections should always be used to supplement each other as '*neither alone can give sufficient information about all dental structures*'.

3.3.4.a Histology – Demineralised Sections

Sections through demineralised teeth and jaws can be used to observe all of the stages of dental development as well as the associated soft tissue. Demineralised histological techniques allow immature enamel to be easily observed as it is not dissolved by the demineralisation process required to produce such a section; instead it is stained, usually dark purple by haematoxylin and so is clearly visible. However, mature enamel is completely dissolved during the demineralisation process, with the surrounding soft tissue producing a replica of the former enamel surface; this is why mature enamel is observed using ground sections.

Like radiographs it is possible to study dental development by comparing corresponding areas of the jaws of infants of different ages; however the advantage of histological sections is that there is no superimposition of structures, which is unavoidable in a similar radiographical study and so the final picture is much clearer. However, as with all cross-sectional studies there are of course individual variations amongst the infants from whom the specimens are obtained.

Logan and Kronfeld (1933:424) stated that the use of histological sections provided definite evidence of the '*state of development and calcification of each germ*'. They went on to say that the advantage of using serial sections in dental developmental research as opposed to single sections, is that some important structures are so small they are not actually visible at the time of sectioning and that these small structures only become visible after the sections have been mounted and stained. Such information may be lost in single histology sections or even if each individual successive section is not examined in turn.

In the serial section method used by Logan and Kronfeld (1933) and Logan (1935) the jaws were dissected from the rest of the body within a few hours of death and fixed in formalin and alcohol. They were then demineralised so that all of the mature enamel was dissolved and of the bone and dentine only the

organic matrix was left. The jaw was then embedded in celloidin and sectioned in series; four planes of section were used (frontal, sagittal, horizontal and labiolingual or buccolingual). The sections were then stained with haematoxylin and eosin in order to allow the developing tooth germs to be observed more clearly. In their 1933 study each serial section was about 35µm thick, the largest sections produced were 8cm in length by 6cm in width (Logan and Kronfeld 1933).

The use of serial sections also allows the structure being examined to be reconstructed. Histological sections present in two-dimensions, however having a series of consecutive sections allows a three-dimensional picture to be built up.

Although this method of examining crown formation proved to be considerably more accurate than radiography (Kronfeld 1935a; Kronfeld 1935b; Kronfeld 1935c; Logan 1935; Logan and Kronfeld 1933), very few similar studies have been performed since, (Calonius et al. 1970; Sunderland et al. 1987; Turner 1963).

3.3.4.b Histology – Ground Sections

Kronfeld stated that two methods can be used to prepare ground sections, the first by hand and the second using a grinding machine. Evidently according to Kronfeld (1937:172), '*the grinding of teeth can be learned without much difficulty by anybody possessing average manual dexterity*'.

The method Kronfeld advocated when grinding by hand was that the teeth were first cut into 1-2mm thick sections using a carborundum or steel disc and a dental hand-piece. Then both sides of the section were ground down in turn, on polishing stones of decreasing coarseness. The section has to be kept constantly wet, either with water, alcohol or glycerine, in order to stop it drying out and cracking during preparation. The last grinding was done using fine wet

pumice powder on a glass slab; finally the section was polished using aluminium oxide, washed thoroughly and then mounted.

Although ground sections only allow the hard tissue to be studied, as the soft tissue is either lost or distorted during preparation, they are '*indispensable*' for the microscopic examination of enamel (Kronfeld 1937:173). Ground sections were produced as part of the histological section of this thesis.

3.3.4.c Histology – Early Incremental Studies Using 'Tooth Ring Analysis'

Schour and Poncher (1937:764) argued that the time of formation of any given incremental layer is '*in proportion to its distance from the dentino-enamel junction*' and in 1941 Massler et al. attempted to define a number of 'growth rings' which each marked a specific stage in normal crown development. This method is comparable to that employed in tree ring analysis (dendrochronology) and is therefore termed 'tooth ring analysis'. These 'growth rings' were supposed to correspond to a specific period in juvenile development. For the deciduous enamel crown these were the 'prenatal period', which was demarcated by the neonatal 'ring' and then the 'infancy period', which was demarcated by the infancy 'ring'. The presence of an additional 'early infancy ring' was also tentatively suggested by Massler et al. (1941:49), this ring was supposedly present in teeth mineralising at about six months and was reported to be present in the enamel of 60% of deciduous second molars.

Massler et al. (1941:49-50) reported that the 'infancy ring' was a '*sharply accentuated incremental line*' that could be observed in the enamel mineralising at about ten months, although the '*absolute time and the position of the ring in the teeth may vary slightly*'(1941:49). The 'infancy ring' was reported as being a '*hypocalcified line*' that was present in the dentine in 75% of deciduous teeth (1941:51-52). Due to the completion of most of the deciduous enamel crowns by this time, only these three 'rings' are relevant to this current work, although Massler et al. (1941) identified five 'rings' in total. However, with the exception

of the neonatal line which has been firmly established as occurring at birth, the presence of these additional ‘rings’ has not survived the test of time, although this work did contribute to the development of the deciduous crown formation chronology.

The numerous studies developed by Schour using ‘tooth ring analysis’ with the neonatal line as its basis, can be used as a method to establish the age of initial mineralisation for deciduous enamel in utero, without the requirement of fetal specimens (Schour 1936a; Schour and Kronfeld 1938; Schour and Massler 1937; Schour and Poncher 1937). This method therefore is not influenced by the aging problems encountered by the previous methods discussed. This method involves using the neonatal line as a chronological landmark of birth (day 0); by measuring the greatest amount of prenatally formed enamel between the neonatal line and the EDJ back along the direction of the enamel prisms (the path along which growth occurs) and then by dividing this distance by the daily apposition rate, the number of days taken to form the prenatal enamel can be calculated. This number of days before birth is equal to the time of enamel initiation.

In the same way, postnatal enamel formation times can be calculated by measuring the distance between the neonatal line and an accentuated line further into enamel formation. This method requires that measurements must always be taken along the prism path. Once the daily apposition rate is known, the number of days taken to form the postnatal enamel can be easily calculated. However for this method to work it is essential to know the daily rate of enamel formation in the region that is being examined.

When using the neonatal line as a chronological landmark of birth, the time of enamel formation in the crown can be calculated for both prenatal and postnatal enamel, although it must be remembered that if using such a method to age infant remains, it is the age of the tooth that is being established not the age of the individual. Aging juvenile remains using ‘tooth ring analysis’ is limited by the length of time it takes to form the enamel crown, once the enamel has stopped

forming it can no longer be used to determine the age of the individual it belonged to. This is because the biological enamel clock 'stops' at the point that the enamel crown becomes fully formed and root formation commences; therefore this method no longer estimates the age of the individual and instead will only estimate the formation time of the crown.

3.4 The History of Deciduous Crown Chronologies

The following section reviews the history of how deciduous crown initiation and completion times have been derived and documented. It outlines the various methods used and presents the data reviewed as five summary tables. These tables represent a comparative database with which the results of the current histological study have been compared in **Chapter 8**.

One of the earliest references in the literature to the mineralisation of the deciduous dentition was published in 1861, this was the result of a lecture given by Jacobi (1861) entitled '*Lecture On Dentition And Its Derangements*'. According to Jacobi (1861:401) the '*osseous development of the teeth*' commenced at the fifth month of fetal life. Jacobi (1861:402) stated that the order of dental development '*depends on the general rule of solidification in the foetal body, which begins in the median line and progresses to either side simultaneously*', however, in his next sentence he stated '*thus, the inner incisors are formed first, and the posterior molar teeth are formed last, with the exception of the canine, which appears later*'. Jacobi (1861:402) also added that mandibular teeth develop before maxillary teeth '*in correspondence with the earlier ossification of the lower jaw in foetal life*'. Unfortunately, no details are provided regarding the methods used to obtain this data or the sample used.

One method used to observe the development of the soft tissue of the dental follicle and to demonstrate the process of crown formation of the deciduous dentition is dissection and one of the earliest dissection studies of tooth germs was carried out by Robin and Magitot (1860-1863). Originally published in

French, this research was presented in 18 parts in the *Dental Cosmos* running from 1860 to 1863. Using dissection and transmitted light microscopy these researchers observed dental development in human fetal specimens and several other domestic species and they compiled a very detailed description of the entire process. From a '*series of microscopic preparations*', Robin and Magitot (1861:643) recorded the appearance of '*the first cap of dentine which appears in each follicle*'. Although these times are recorded as the appearance of dentine rather than enamel, Robin and Magitot (1862:4) went on to say later in their work that '*enamel begins to show itself at the summit of the dentine cap at the period when the cap measures about one millimetre in total height'*, which would considerably increase their reported times for the initial appearance of enamel. In their text there is a slight discrepancy in the initial mineralisation time of the second molar, a time of three weeks after the appearance of dentine in the first molar is given (108–113 days) as well as a time of 15 days after the appearance of dentine in the second incisor (109-114 days), for this reason the time of initial mineralisation of the second molar appears in **Table 3.3** as 108-114.

Robin and Magitot (1861:645) suggested that the maxillary teeth are less advanced than the mandibular teeth '*by some days at most*'; particularly in the case of the second molar, where dentine is absent from the maxillary tooth even though it is present on the mandibular tooth at 120 days.

The methods and techniques used by Robin and Magitot (1863) are described in detail in the final chapter of their work, although they appear to have dissected fresh fetal material they do not present any details regarding their sample, its size or how it was aged.

In 1880 Legros and Magitot, published '*The Origin And Formation Of The Dental Follicle*'. This work was again translated from French. In the preface, the translator stated that in their previous work published in the *Dental Cosmos* the '*portion devoted to the origin and formation of the dental follicle was in many*

respects incomplete, and in some particulars erroneous' (1880:3), however, with improved techniques and increased experience the current authors had been able to correct, update and improve this research.

As in the 1860-63 work published by Robin and Magitot, this work presented an in depth description of the development of the dental follicle, this time a table was presented which included a '*Chronology Of The Dental Follicle In Man*' (1880:160), again only the '*periods at which the dentine-cap first appears*' are given. These dates, although still earlier than those presented by other researchers are significantly later than those that were first published by Robin and Magitot (1860-1863). In their text the authors reported their first observation of '*a cap of embryonal dentine*' (1880:153) in the incisors and canines as occurring at 16 weeks and for the first and second molars at 17 weeks, however in their table, initial mineralisation was reported as 17 weeks in the maxillary canine and 16 weeks in the mandibular canine (1880:160). Forty years later Noyes (1921:329) published a tabulated version of this data (maxillary canine 17 weeks) in his text book '*Dental Histology And Embryology*'.

Legros and Magitot (1880:147) examined the developing dentition of a '*large number of embryos*' both of human and domestic animals, although the exact number is not reported. The ages of the human specimens, '*in the absence of positive evidence as to the period of conception, must, in most cases, be determined by measurement*' (1880:147). The human embryos ranged from three to 37cm in length and from these measurements, their ages were estimated to range from the 7^{th} to the 28^{th} week of intrauterine life.

In 1877, Peirce published a table of '*The Development Of The Teeth*', which consisted of a '*presentation of the results of the investigations of others*' (1877:399) and included the '*appearance of the cap of dentine and enamel*' (1877:400). Peirce provided no further information regarding the data source of this table, although this information may have been derived from the work of Magitot (1874) as these values are identical to those provided and cited by

Tomes (1889) and which were adapted from Magitot's 1874 work. Unfortunately this original source could not be located.

In 1884, Peirce presented a pictorial chart of dental development, although he stated that '*in dating, the progressive solidification of tissues, we can with a degree of certainty, mark the beginning and the end only*' (1884:449), he only presented the initial mineralisation times. In his text, Peirce then contradicted his chart by stating that initial mineralisation in the molars commences by the '*end of the nineteenth week*' (1884:451) and not the 18th week. Again there is no indication where this data originated from, but it is possible that it was the same data he used in his 1877 paper.

In 1894 Bödecker published Peirce's table in his text book '*Anatomy And Pathology Of The Teeth*' in a chapter detailing '*Faulty Development*' (1894:194) in which he discussed the chronological position of enamel hypoplasia. This same 1884 chart was reproduced again 32 years later, twice by Brophy in Volume One of '*Oral Surgery*' (1916b:356), curiously in relation to the eruption of the teeth, even though this table does not present eruption times and again in the same year in Volume Two (1916a:787) in relation to the extraction of teeth. Peirce's table was still being cited in 1931 by Swanson in his investigations of the age-incidence in enamel of Retzius lines (1931a) and in his work on the relation of growth velocity and the quality of enamel (1931b).

Tomes in his 1889 edition of '*A Manual Of Dental Anatomy*' included a developmental table titled '*From Magitot. Comptes Rendus 1874*' (1889:154-155). As in previous work by Magitot, the time of appearance of the dentine caps was recorded. The ages of these specimens appear to have been estimated by crown-heel measurements as an age conversion column has also been included in this table. There is no further explanation regarding the origin of this table and there is no further reference or information about the 1874 work by Magitot. This is interesting as the work by Legros and Magitot in 1880 is later than that presented by Tomes, who has cited Magitot's 1874 work. However, in

the seventh edition of this book, Tomes stated that in his former editions he had presented Magitot's table giving the time of the appearance of various dental structures, but that in this and his last edition, he refers to Röse's table which was, in his opinion more reliable. Tomes (1914:213) commented that the age of Röse's specimens, which had also been determined by fetal length were more accurate than the ages of some of Magitot's specimens. However, Gantz (1922) stated that in his study, two of his mandibular radiographs '*seem to indicate that Magitot's table is more nearly correct*'. In Tomes 1914 table, which is titled '*Tables Of Dates At Which The Several Structures Appear In Human Embryos, Adapted By Röse*' (1914:214-215) only five specimens, aged 17 weeks to 33 weeks were presented to illustrate deciduous dental development. Unfortunately as Röse's, work is in German and no translation is available, this work has not been included in this thesis.

Broomell and Fischelis, in their fourth edition of '*Anatomy And Histology Of The Mouth And Teeth*' published in 1913, presented the results of their dissection and microscopic examination of the developing dentition of the human fetus. They dissected tooth follicles from their crypts, together with the overlying mucous membrane and periosteum. By further dissecting the delicate mineralised caps from the underlying pulp tissue they were able to follow the progress of mineralisation at different ages. Broomell and Fischelis (1913:219) stated that the mineralisation of deciduous teeth was similar to that of the permanent teeth, it commenced in the incisors and canines at the incisal edges and occlusal tips '*in three distinct lobes*', while in the molars '*a centre of calcification is provided for each cusp*' (1913:219). They presented their initial mineralisation times in association with a photograph of each tooth type (1913:219-230), they did not present any crown completion data, except to say that '*by the beginning of the second month after birth, calcification in the crowns of all the deciduous teeth is about complete*' (1913:432). They also reported that there was '*a slight variation*' (1913:419) between the maxillary and mandibular teeth which was present in nearly every instance, with the mandibular teeth developing ahead of the maxillary teeth.

Although Lunt and Law (1974:600) stated that '*the size of the sample examined was not given*' and this is in fact the case for the main text, in their preface Broomell and Fischelis (1913:ix) indicated that '*about 100 dissections*' were carried out. Although they presented no further information about their sample or how it was aged, Gantz (1922:131) suggested that the '*age determination of some of the younger specimens seems quite open to question*'. On comparing the published work of these two researchers this comment does appear to be justified, this is further supported by the examination of a photographic plate that Broomell and Fischelis had included in their work which illustrates '*deciduous teeth at birth*' (1913:Fig 331:424) and which shows a nearly completely mineralised series of deciduous teeth, with the molars with '*their crowns calcified to about one-half their completed length*' (1913:424).

In 1922, Gantz produced a set of illustrations and radiographs depicting fetal jaw development with the teeth in situ; this work was the result of dissection and the observation of fetal mandibles cleared with alcohol and cedar oil, in older fetal specimens (aged between five months and birth), potassium hydroxide was used to assist clearing. Gantz was one of the first researchers to study mandibles with the teeth in situ using such a clearing method. He also dissected the corresponding maxillae of the cleared mandibles and concluded that the '*development was about the same in both jaws*' (1922:132). These studies were further supplemented with radiographs. This sample consisted of 50 fetuses preserved in 10% formalin and 23 dried skulls. The majority of the formalin preserved specimens were also injected with 10% formalin via the umbilical vein at the time of fixation. The age of these fetuses was determined by crown-heel length measurements. Although Gantz was one of the first researchers to actually present detailed information about his sample and experimental method, he does not present any specific times for either initial mineralisation or crown completion, just general descriptions. However, he does state that at birth the cusps of the first deciduous molar have coalesced '*forming a solid occlusal surface*' (1922:137), and that the cusps of the second molar are mineralised but are still separated from each other. Previously in 1908, Symington and Rankin in their '*Atlas Of Skiagrams Illustrating The Development Of The Teeth, With Explanatory Text*' had stated that the '*multicuspidate teeth*

begin to calcify by independent deposits on the apices of prominences on the dental papilla, and the separate cusps thus formed are gradually united over the surface of the less projecting portions' (Symington and Rankin 1908:2). Symington and Rankin also confirmed this with the inclusion of radiographic prints in their work, illustrating that at birth the cusps of the second lower molar are not yet completely united, although unlike Gantz, Symington and Rankin reported that in their newborn specimen, four of the cusps had united and only the mesial-lingual remained isolated.

In 1924, Mummery in his second edition of '*The Microscopic And General Anatomy Of The Teeth*' presented a '*Table Of The Dates Of Appearance Of The Several Structures In Human Embyros*' which had been '*adapted from Röse and C. S. Tomes*' (1924:27). No details are given regarding why this adaptation was required and there is no further information describing this sample, however it does appear that the ages of the embryos have been derived from fetal length as these lengths have been included in this table. Mummery also described the state of development of the deciduous dentition at birth.

In 1924, Brady published '*A Chart Of The Average Time Of Development, Eruption And Absorption Of The Teeth*' this self-published 15 page booklet was aimed at dentists and parents. Brady reported that by the 17th week of embryonic life the '*three developmental points*' of the incisors have begun to mineralise and by the 20th week the first and second molars begin to mineralise, the first molars from '*four points*' (1924:6) and the second molars from four for the upper and five for the lower teeth. These soon coalesce and by the 25th week they form a '*very thin but continuous shell over the grinding surface*' (1924:6). Brady stated that the crowns of the incisors advance faster than the crowns of the molars, he suggested that this was because they '*start earlier and develop faster*' when compared to the molar crowns (1924:6). Brady (1924:5) did not give any details regarding the sample he used to create his chart, apart from stating that his chart was the '*result of more than twenty-five years' study*'.

In 1932, Churchill presented a table of the '*Evolutionary Phases of Dental Development*' in the appendix section of his book '*Human Odontography and Histology*' (1932:170); here he listed initial mineralisation times and the degree of mineralisation at birth, unfortunately he did not present any crown completion times. Churchill also stated that development in the mandible usually preceded that in the maxilla. There are no details regarding how these times were obtained, Lunt and Law (1974) suggested that maybe histological methods had been used, as prints of histology slides appear in the text, however there is no mention of any sample and the origin of this table is unclear.

In 1932, Hess et al. studied crown development using both radiography and dissection. They produced a series of radiographs that clearly demonstrated developing tooth germs within the jaws of living healthy juveniles, with ages ranging from birth to adolescence. The exact sample size was not given. Hess et al. (1932) raised the point that radiographs and anatomical dissections do not produce similar results when observing crown formation and they stressed the point that mineralisation always appears much further advanced in anatomical dissections than it does in radiographs of the same specimen. They found that upon dissection, the crowns of deciduous incisors at birth were found to be mineralised to about two-thirds of their final extent; however, in radiographs they appeared as only being mineralised along their incisal edge and for about two-thirds of their lateral borders. It is the extent of this mineralisation along the lateral borders that Hess et al. (1932) suggested provides the correct information as to the total area of mineralisation of the crown.

Although the age range of this sample does mean that fetal crown development was not examined, Hess et al. (1932) described the degree of mineralisation of the deciduous dentition at birth. They concluded that based on their anatomical and radiological studies, the majority of the mineralisation of the deciduous teeth '*takes place subsequent rather than previous to birth'* (1932:1058).

In 1935, Wolfe published his observations of '*Teeth In Fetal Rickets*' and he included a descriptive text and a chart '*representing the calcification period of*

the deciduous teeth' (1935:907), however there is some discrepancy between the two. The chart indicates that the incisors commence mineralisation at 20 weeks and the molars and canines 24 weeks. It appears that this chart may have originally been produced by Tomes from work he had published in 1923 and indeed these times are the same as included in Tomes 1914 table (after he had updated it due to errors in fetal age estimation), unfortunately no further details are provided. However, in his text Wolfe stated that mineralisation normally begins in the incisors at 17 weeks in utero and he then proceeded to use 17 weeks in his remaining text as the time of incisor initial mineralisation.

'*Meyer's Histology And Histogenesis Of The Human Teeth*', translated from German by Churchill in 1935, presented a very detailed description of deciduous dental development including diagrams, models and photomicrographs, which had been produced from dissections and serial sections. Again the mandible was reported as being in a more advanced stage of development than the maxilla. Although no details about this sample are presented in the main text, in the preface Churchill (1935:v) stated that in order to illustrate the consecutive stages of dental development '*one hundred embryos were sectioned or dissected*'. It appears that crown-rump measurements were also used to age these specimens as a crown-rump length conversion table is included at the start of Part Two of this book which then proceeds to describe histogenesis. However, from the hundred embryos studied, only 12 are listed in the '*Tabulated Review Of Tooth Development*' (Churchill 1935:291) additionally this table has also been '*modified after Röse*' although no description has been given as to how or why this work has been modified. Initial mineralisation times and crown completion times are also reported in this table; although the degree of crown formation at birth and the crown completion times are not complete.

This work had been translated and edited by Churchill who also included a copy of his own 1932 table, which he re-titled the '*Phases Of Odontogenesis Tabulated Chronologically*' (1935:296) and which has been included '*for the purpose of comparison*' (1935:297). Churchill also added that when using tables

that present a chronologic review of dental development, it must be remembered that the material used to produce such a table is subject to individual differences. He also stated that for '*obvious reasons a large amount of post-natal material for dissection is difficult to obtain*' (Churchill 1935:297).

In 1933, Logan and Kronfeld, radiographed and then serially sectioned 25 complete juvenile jaws that had been removed during post-mortem examinations. Of the 25 jaws examined, ranging in age from birth to 15 years, only 16 were from children under the age of one year, therefore as a consequence of this age range, this work concentrated mainly on the permanent dentition and only very limited descriptive information was presented for deciduous crown formation.

From their observations of three specimens (one of who died of debility from a cleft palate), Logan and Kronfeld reported that in the newborn, mineralisation of the incisal edges and tips of the cusps was visible, with the cusps of the second molars, which were not yet united, appearing as separate centres of mineralisation. At the age of nine months, from observations of just one specimen, (who probably died suffering from rickets (Kronfeld 1935a)), Logan and Kronfeld (1933:410) reported that '*calcification of the crowns of all deciduous teeth seems to be complete*'. They also stated that as a rule '*the lower teeth are somewhat ahead of the corresponding upper teeth in their development*' (1933:414) and they presented histological sections to support this statement (1933:391 and 400).

Many of Logan and Kronfeld's (1933) specimens had died from tuberculosis or associated diseases such as empyema pleurae, meningitis etc. Some died of severe intestinal disturbances (enteritis), one died of diphtheria, one of scarlet fever and several of the very young children died of '*debility*' (1933:394) including two who had cleft palates. From their radiographic studies, Logan and Kronfeld (1933:394-395) concluded that '*there was by no means a constant ratio between the degree of calcification and the age of the child*' and they suggested that this may partly be '*due to the fact that in some of these children,*

development was retarded by prolonged illness' (1933:395) and in other cases it may be the '*result of individual variations within a physiologic range*' (1933:395). There are wide variations in the development of the human body, even in healthy children and consequently more so in sick children who finally die of the disease that they were suffering from. Because of this wide variation in body development Logan and Kronfeld were more concerned with identifying the sequence of mineralisation rather than its exact timings, which they stated are naturally subject to wide individual variation and which are exaggerated in this case by the pathological nature of their sample.

In addition to the pathological nature of this sample, which undoubtedly affected the resultant crown formation times, Logan and Kronfeld (1933:393) also stated that they '*do not consider the absolute ages*' of their '*specimens the most significant item*', a statement which may also bring the ages of their sample into question.

Although the sample size used to observe deciduous crown formation at birth, only consisted of three specimens and the resultant descriptions are incomplete and in need of refining, this was one of Logan and Kronfeld's (1933) first attempts to create a chronology of deciduous crown formation using serial sections and radiographs.

In 1935, Logan added another two specimens to the sample that he had used in 1933, bringing the total sample size up to 27 juveniles ranging in age from birth to 15 years of age, both of these new specimens were in the under two age range (probably 'newborn' according to Kronfeld's 1935b work). Logan claimed that approximately 10,000 sections through infant jaws had been prepared over the past four years. However, despite this large number of sections, this work again concentrated mainly on the permanent dentition and only very limited descriptive information was presented for deciduous crown formation.

In 1935c, Kronfeld summarised the work that Logan had begun in 1929, this time indicating that an increased sample size of '*more than thirty human jaws,*

ranging in age from birth to 15 years' (1935c:18) had been utilised, no further details are provided regarding this new enlarged sample. In this work Kronfeld presented a list and a table of deciduous crown initiation and completion times and he added that these initiation times and the sequence of mineralisation had also been corroborated by the observation of the distribution of hypoplastic defects as well as by radiographic studies. Kronfeld (1937:123) stated that in the production of this table, mineralisation was considered to have commenced when '*the small cap of dentin and enamel first appeared on the tooth germ*'. Enamel formation was considered to be complete when the '*enamel had been formed to the cemento-enamel junction, the crown had attained its final shape, and there was no evidence of activity of ameloblasts*' (Kronfeld 1937:123-124). Kronfeld (1937:110), stated that dentine formation always begins a '*short time*' before enamel formation and that the earliest evidence of both dentine and enamel formation was observed in the deciduous incisors during the sixth month in utero (although he presented this as five months in his table).

Interestingly, none of the material from the joint work with Logan in 1933, or Logan's 1935 work or even Kronfeld's 1935a and b work detail any fetal specimens. So the actual source of the material used to develop this chronology of deciduous development is unknown. In 1935 Logan had increased the original sample to 27 (ranging from birth to 15 years), so at least two of the new 1935c specimens had to have been fetal specimens (aged five months and six months in utero) as in the 1933 and 1935 studies the youngest specimens were newborn. Two other 1935c specimens that must have been added to the new sample in order to produce this table, had to have been aged four months and ten months old, as again these ages are missing from the original 1933 and 1935a and b samples. It seems very likely that the sample size used to produce this table probably consisted of only one individual for each of the eight different ages included in this table; this would mean that a minimum of two fetal specimens and six infant specimens, aged from four months to 12 months were used to create this table.

In 1937 Kronfeld included the table that he had published in 1935c, in his book '*Dental Histology And Comparative Dental Anatomy*' (1937:129). Several plates of fetal histological specimens were included in this work, including sections at five and six fetal months, these are the missing fetal ages from the 1935c table that were not in the original 1933 and 1935a and b samples. So it seems possible that these are the two specimens that were used to produce the table published in 1935c. It also appears that the ages of this fetal material may have been derived from fetal length measurements, as these have also been included with several of the photographic prints.

In March 1935, Schour and Hoffman submitted two Abstracts (1935a and b) to the 13th General Meeting of the International Association For Dental Research. They had been investigating the incremental growth of teeth. In their first abstract, using both ground and demineralised sections they measured the widths between the '*pairs of light and dark incremental layers that normally constitute the stratification of enamel and dentin*' (1935a:161). These incremental layers were the striae of Retzius in enamel and the Andresen lines in dentine. These layers, Schour and Hoffman stated, were of '*physiologic significance because they reflect the nutritional and metabolic variations that occur during the growth and calcification of the dental tissues*' (1939a:91) and as no remodelling occurs in the teeth after they have mineralised, records such as this are permanent.

Using 34 ground sections (the enamel had been dissolved in the preparation of the demineralised sections), Schour and Hoffman measured the width between successive striae of Retzius, they took 465 measurements and established that in human enamel the mean value between successive striae is 15.87µm (standard deviation of ±2.87 and a mean error of 0.230). From another sample consisting of 183 teeth from 17 different species, Schour and Hoffman (1939a) concluded that the mean value of the width between each successive incremental layer was approximately 16µm. They reported that there was no significant difference in the width between these incremental lines in either enamel or dentine, between different tooth types or between different species.

Although no specific reference is made to the deciduous dentition in the main text of this work, a brief mention was made in the discussion which stated that deciduous teeth also share this 16µm rhythm.

Schour and Hoffman (1939a:100) concluded that the pairs of light and dark layers which recur at intervals of 16µm and which have an incremental pattern '*reflect a basic physiologic rhythm in calcification*'. They suggested that as a result of systemic disturbances affecting calcium metabolism, the incremental line forming at the time may become accentuated (i.e. stria of Retzius or Andresen line). This accentuation of the normal increments may be produced by '*constitutional or environmental factors*' (1939a:100) such as birth (Schour 1936a) or by pathological conditions that disturb calcium metabolism (Schour 1938).

In their second abstract Schour and Hoffman (1935b) reported that by the use of timed experimental injections of sodium fluoride into animals and the production of ground and demineralised sections, they were able to calculate the daily rate of enamel apposition. Schour and Hoffman (1935b:161) discovered that each injection of sodium fluoride '*produced a prompt record in the form of an undercalcified layer in the portion of the dentin or enamel that was then being apposed*', i.e. apposed at the time of the injection. By measuring the distance between two of the successive 'undercalcified layers' and by dividing this distance by the number of hours between the two successive injections, the rate of apposition could be ascertained. For the enamel of albino rats this was found to range from 15.70-16.37µm per twenty-four hours (1935b). Schour and Hoffman (1935b:162) concluded that the finding that '*16 micra represent the amount of daily apposition of enamel and dentin indicates that the 16-micra rhythm observed in the normal incremental stratification is a 24-hour phenomenon*'. In 1937 and 1939b Schour and Hoffman confirmed this daily 16µm rate of apposition for rat dentine using alizarin red S (sodium sulphalizarate) (1937:349) instead of sodium fluoride. Schour, this time working with Poncher (1937:760) also confirmed this daily 16µm rate for '*smaller*

animals' in their 1937 work, when they also presented a daily apposition rate for humans.

In 1939b when this work was published in full, Schour and Hoffman had observed the rate of apposition in a total of ten different species using both sodium fluoride and alizarin red S injections. They had also added *Macaca mulata* rhesus monkeys to their sample and had obtained an average daily rate for dentine apposition of 4µm; a gradient effect, similar to that found in humans (Schour and Massler 1937; Schour and Poncher 1937), was also reported for *Macaca* and this ranged from a daily rate of 2.4µm in the cervical region to 12.2µm in the occlusal region.

The 16µm mineralisation rhythm for the enamel and dentine of different species established by Schour and Hoffman (1939a) persisted in spite of the differences in the daily rate of apposition between the species studied. Schour and Hoffman (1939b:171) suggested that this was due to the fact that the process of mineralisation was a '*physico-chemical precipitation of calcium salts*' and therefore, it was essentially similar in all of the species that they studied. While the process of apposition they suggested, was dependent upon the activity of the formative cells and therefore tended to vary among different species. This constancy of the 16µm mineralisation rhythm, remained unaltered even by severe experimental interferences such as parathyroidectomy or hypophysectomy (Schour and Van Dyke 1932).

Although the title of this work is '*The Rate Of Apposition Of Enamel And Dentin In Man And Other Mammals*' (Schour and Hoffman 1939b), the reader is referred to the work of Schour and Poncher (1937) for the rate of apposition in man.

In 1937, Schour and Poncher had the opportunity to quantitatively determine the daily appositional rate of deciduous enamel formation in human teeth. They

injected 25 small doses of sodium fluoride at known intervals, for a period of 130 days, into a five month old male infant with inoperable hydrocephalus and meningocele. When the child died at nine months as a result of confluent bronchopneumonia, his mandible and upper incisors were subjected to radiographic and histological examination. One ground section of the deciduous maxillary central incisor was prepared and the remaining teeth were demineralised and serially sectioned. Schour had previously demonstrated with Hoffman in 1935b that each sodium fluoride injection resulted in the appearance of a sharply accentuated incremental line in the enamel and dentine which had been forming and mineralising at the time of the injection. Schour and Poncher also reported that the direction of the enamel prisms appeared to be '*stunted and interrupted after each injection*' with this resulting '*waviness and twisting of the rods*' indicating that a disturbance in formation had occurred (1937:769), they also added that the line consisted of '*an imperfect, hypocalcified layer followed by a secondary recovery reaction which was evidenced as a normal or hypercalcified layer*' (1937:758).

By injecting the infant at known times, Schour and Poncher were able to calculate the average rate of apposition by measuring the distance between successive lines of fluorosis along the direction of the enamel prisms and dentine tubules (as this is the path along which growth occurs) and dividing this measurement by the number of hours between two injections. Unfortunately, in the deciduous teeth most of the enamel had fully formed and mineralised before the first injection was given at five months and so many of the later injection lines did not appear in these sections. In addition, in the serial sections of the deciduous teeth, most of the enamel was fully formed and mineralised by the end of the nine months and as a result of this, only a small cervical area of enamel remained in the mandibular second molar after the demineralisation process. However, Schour and Poncher were able to identify 12 injection lines and obtain a limited number of measurements (165) in this area. From these 165 cervical enamel measurements they calculated that the daily apposition rate ranged from 3.6 to 4.3µm per twenty-four hours, with the total average being 3.92µm per twenty-four hours (standard deviation of 0.26 and a probable error of 0.01 micron).

From their measurements Schour and Poncher (1937) identified the existence of a slight but consistent gradient in the average rates of apposition. This was between the different regions of the tooth, where the apposition rate decreased consistently from the occlusal to cervical enamel and which Schour and Poncher suggested appeared to correspond to the form and contour of that portion of the tooth. Schour and Poncher also stated that their observations between the ages of five and nine months '*do not indicate any intervals of remission or rest in the formation of enamel*' (1937:774) and that this period '*may be one of the active and continuous periods of growth*' (1937:775).

Although the enamel in the ground section of the deciduous maxillary central incisor showed no effects from the injections, as it had completely formed and mineralised before the injections were given at five months, it did exhibit a neonatal line and Schour and Poncher were able to demonstrate that this line was produced on the day of birth. By identifying the corresponding neonatal line in the dentine they measured the distance from this line to the first injection line in the dentine and then they divided this distance by 4µm (the approximate average daily rate of dentine apposition in that region) this calculation resulted in the number of days that had elapsed between the formation of the line in question and the first injection line, this day was identified as being the infants birthday. This, therefore, proved quantitatively that the neonatal line was definitely associated with the event of birth. Schour and Poncher also stated that the enamel that had formed prenatally appeared to be better mineralised than the enamel that had formed postnatally.

Schour and Poncher (1937:772) observed changes in the ameloblasts which exhibited a number of '*fine hematoxylin-staining globules*'. They suggested that these globules may have been an effect of the last injection and that they may be comparable with those they had observed in the ameloblasts of rats which had been killed within one to twelve hours of a single sodium fluoride injection, (Schour and Hoffman 1939b; Schour and Smith 1934a; Schour and Smith 1934b), they also added that the primary effect of acute fluorosis in the human

was probably on the ameloblasts, just as it was in the rat. Schour and Poncher (1937:772) observed that enamel hypoplasia, consisting of an arrest in the formation of enamel and '*cysts in the enamel epithelium*' was also present in the infants developing anterior permanent incisors. Although these changes were not severe, Schour and Poncher (1937:772) suggested that they were '*probably associated with either the injections of fluorine or the hydrocephalic condition*'. This last comment seems misplaced as the whole idea of the injections was to produce a hypoplastic event and presumably the rats Schour had observed previously had not suffered from hydrocephalus; however, this comment does raise the issue that maybe the hydrocephalic condition could have been responsible for this resultant hypoplasia. Interestingly, similar '*distinct hematoxylin-staining globules*' were also reported as being present in the cytoplasm of the ameloblasts of some experimental rats that had been subjected to bilateral adrenalectomy (Schour and Rogoff 1936:340).

From one ground section of a maxillary central incisor and one partially demineralised section of a second molar Schour and Poncher (1937) were able to demonstrate that the neonatal line was produced at birth and that the average daily appositional rate of cervical enamel was 3.92µm. They also concluded that the sharp bending of the enamel prisms that they had observed occurring at the time of the injection and the presence of hypoplasia indicated that an arrest in enamel formation had occurred in addition to a disturbance in mineralisation.

It is in this work that the term '*neonatal ring*' is first introduced (Schour and Poncher 1937:772), as in a transverse section accentuated striae and the neonatal line appear as concentric rings '*similar to those seen in trees*' (1937:758), see **Chapter 4**.

In the same year, Schour and Massler (1937) investigated the rate and gradient of dentine apposition. Using ground and serial sections from the jaws of eight children, exhibiting little or no dental pathological disturbances, they used the

neonatal line to demarcate the limit of the pre- and postnatal dentine; then by measuring inwards, along the path of the tubules from the neonatal line to the pulpal surface and by dividing this distance with the age in days of the child at death, they established the apposition rate. Using this technique they presented a table illustrating the variation in the rates of deciduous dentine formation with regards to the region of the tooth and the tooth type. However, the resultant postnatal daily rates of dentine apposition are very high, for example 5-8µm per day for the central incisor when compared to the 3.16-4.42µm results previously obtained for the same tooth type by Schour and Poncher (1937:765).

In 1938 Schour and Kronfeld referred back to this work stating that this 1937 work recorded the average rate of enamel apposition as being 4µm per day (1938:473), however, this is not the case, as the ranges for dentine formation in the deciduous dentition in this work range from 4-8µm (Schour and Massler 1937:350).

Although Schour and Massler stated that only teeth that had actively functional pulps at the time of death were measured, in order to ensure that the dentine was still developing, measuring between two accentuated striae may produce more reliable rates than measuring from the neonatal line to the developing pulpal surface. In addition the position at which these measurements are taken will also affect the results, unfortunately no further details regarding this were provided in this work. Although these apposition rates are presented for dentine, Schour and Massler stated that previous experimental evidence in both animals and humans indicated that the rate of enamel formation approximates that of dentine.

Despite the differences reported in apposition rates between these two studies, the table presented by Schour and Massler (1937:350) does illustrate a consistent gradient of the average rate of apposition in different regions of the tooth. There is a gradient that decreases consistently from the occlusal surface to the cervix in each tooth type and which Schour and Massler (1937:350)

suggested '*conforms with anatomy of the tooth*'. A decreasing gradient from the anterior to the posterior teeth was also proposed (see **Section 8.1**).

One very interesting point that is briefly raised in this work is that Schour and Massler (1937:350) subtract 14 days from their calculations '*to allow for neonatal arrest in growth*'. This subtraction was the result of an additional examination of the jaws of ten children, one hour to six months old and which evidently illustrated that this '*neonatal arrest in growth averages 14 days*' (Schour and Massler 1937:350). No further details were provided regarding this research in this paper. However, it may explain why Schour and Poncher (1937:774) had previously raised the fact that in their observations of the hydrocephalic infant they had not observed '*any intervals of remission or rest in the formation of enamel*'.

These later studies developed by Schour provided a method of 'tooth ring analysis' which is described above (see **Section 3.3.4.c**) and which can be used to establish the age of initial mineralisation for deciduous enamel in utero without the requirement of fetal specimens, as well as for establishing postnatal enamel formation times in developing teeth. This method involves using the neonatal line as a chronological landmark of birth (day 0) and measuring the greatest amount of prenatally formed enamel between the neonatal line and the EDJ along the direction of the enamel prisms (the path along which growth occurs). This distance is then divided by the daily apposition rate and the result gives the number of days taken to form the prenatal enamel. This time can then be used to determine the age of initial mineralisation by counting backwards from birth. Postnatal enamel formation times can be calculated by measuring the greatest distance between the neonatal line and the accentuated line in question or the developing enamel surface. Again these measurements must be taken along the prism path. Then by dividing this distance by the daily apposition rate, the number of days taken to form the postnatal enamel can be calculated.

In 1938, Schour working with Kronfeld applied this method of ‘tooth ring analysis’ to demineralised stained sections and ground sections from the deciduous teeth of a female infant who although born after a normal delivery at term, failed to develop properly or to show a normal response. On admission to hospital with an infection of the upper respiratory tract, she was diagnosed with an injury of the brain that had evidently been sustained at birth. The infant later developed bronchopneumonia to which she succumbed at the age of seven months and five days (218 days).

After taking radiographs, each jaw was divided in the midline, one half was demineralised, sectioned and stained, the other half was made into ground sections. This way it was possible to compare the demineralised sections and the ground sections of corresponding teeth from the same individual. The radiographs showed a state of mineralisation which corresponded to that of a child of approximately six months old. Although born at term and dental development appeared from the radiographs to have been delayed by one month, Schour and Kronfeld (1938:482) stated that in this individual the amount of postnatal dentine was ‘*less than normally would be expected*’ for a seven month old infant and they stated that the dentine development ‘*seemed to be about two months late*’ (1938:482). Schour and Kronfeld (1938:477) also suggested that this juvenile may in fact have been born prematurely due to the higher position of the neonatal line in the dentine. Despite these points Schour and Kronfeld applied ‘tooth ring analysis’ to the dentine to establish the ages at which the teeth of this juvenile initially mineralised, by measuring the distance from the highest point on the dentine neonatal line to the corresponding growth centre on the EDJ the average rate of prenatal dentine formation was determined by dividing this distance by the rate of apposition as described above. The average rate of postnatal dentine formation in this area was taken to approximate the rate of prenatal formation. Schour and Kronfeld (1938:487) defended the use of the same apposition rate for pre- and postnatal enamel stating that ‘*this is valid in view of the fact that the range of variability even under extreme circumstances would be less than twenty days*’ due to the neonatal arrest period. The daily rate of apposition had been presented previously in Schour’s 1937 work with Massler (Schour and Kronfeld 1938:483).

This work gave the rate of apposition for the central incisor as ranging from 5-8µm (Schour and Massler 1937:350) rather than the 4µm daily rate of apposition reported by Schour (1936a:1953) and Schour and Poncher (1937) and even Schour and Kronfeld earlier in the same paper (1938:473). However if the same distance from the tip of the dentine neonatal line to the tip of the pulpal horn (1080µm) is divided by the age of the individual (218 days) then the appositional rate for central incisors should be 4.95µm rather than 8µm, which is more similar to the average 4µm daily rate of apposition reported by Schour (1936a:1953) and Schour and Poncher (1937) and Schour and Kronfeld (1938:473).

Schour and Kronfeld (1938:487) presented the ages that they had calculated using 'tooth ring analysis' for the dentine of this individual in their table '*Beginning Of Dentin Formation In The Deciduous Teeth*'. Although Schour and Kronfeld divided their results table into maxillary and mandibular teeth after mentioning that there are '*slight differences*' (1938:483) between maxillary and mandibular teeth, only the central incisor was different by half a month with the maxillary incisor forming first. As the prenatal dentine was not affected by the trauma caused at birth which resulted in severe postnatal hypoplasia, Schour and Kronfeld (1938:487) felt that these times '*may be taken as representing average normal values*' for all deciduous teeth.

So from just one single individual Schour and Kronfeld (1938) established the ages at which all deciduous crowns initially mineralise.

In 1939, Kronfeld and Schour presented a '*Chronology Of Human Deciduous Teeth*' (1939:21). The origin of this table was not discussed, except to say that it was developed using 'tooth ring analysis'. The only difference between this most recent table and the one that was presented the previous year was that the value for initial mineralisation for the second molar had been increased from five and a half months to six months; no explanation for this change was given. In the previous table, initial mineralisation was presented as the '*Beginning Of Dentin Formation*' (Schour and Kronfeld 1938:487) while in the more recent

table it was presented as '*Hard Tissue Formation Apposition*' (1939:21). Using the neonatal line as a biological landmark, Schour and Kronfeld also presented the '*Amount Of Enamel Formation At Birth*' as well as the time '*Enamel Completed*' which was evidenced by '*preclinical or intraosseous eruption*' (1939:21). However, exactly which specimens were used to generate each section of this table was not specified.

Following the presentation of this table, Kronfeld and Schour referred back to the case study that they had presented the previous year, involving the infant who had sustained a brain injury at birth and from which they had developed their original table the '*Beginning Of Dentin Formation In Deciduous Teeth*' (Schour and Kronfeld 1938:487). They then presented two more case studies of individuals exhibiting hypoplasia resulting from birth injuries and whose medical histories were also available. The mineralisation of prenatal enamel and dentine was then discussed (see **Section 4.5**) and then an additional sample of '*approximately fifty complete jaws of infants and young children*' as well as the '*shed or extracted deciduous teeth of more than 600 additional children*' was mentioned, but whether or not these specimens were used to create the '*Chronology Of Human Deciduous Teeth*' table remains unclear (1939:25).

A year later Schour, this time working again with Massler, presented another table detailing the '*Chronology Of Growth Of Human Teeth*' (1940a:1920). This table is similar to the one Kronfeld and Schour (1939) had published the previous year except that several of the initial mineralisation and crown completion times had now been merged together, however the amount of crown formed at birth was still divided into maxillary and mandibular amounts for the incisors. No reason was given for this partial merging of data. However, the diagram presented with this current work detailed exactly the same times that were presented the previous year. The only information that was provided regarding this table was the fact that it had been '*modified*' (1940a:1920) from the work of Logan and Kronfeld in 1933. However as this work did not contain any fetal specimens, it is unlikely that the fetal data was derived from this particular source. The 1933 study consisted of 25 complete juvenile jaws that

had been removed during post-mortem examinations, radiographed and then serially sectioned. Of the 25 jaws examined, ranging in age from birth to 15 years, only 16 were from children under the age of one year, therefore as a consequence of this age range, this 1933 work concentrated mainly on the permanent dentition and only very limited descriptive information was presented for deciduous crown formation, no data was presented for times of initial mineralisation for deciduous teeth at all in this work. However, it is possible that Kronfeld's 1935c sample contributed to this table as this sample did in fact contain at least two fetal specimens, which must have been present in order to produce Kronfeld's 1935c table. It is more likely that the source of fetal material used to create this table came from Kronfeld and Schour's 1939 study, which used 'tooth ring analysis' to determine the times of initial mineralisation, crown completion and the amount of crown formed at birth, as apart from the merging of some of the maxillary and mandibular data both of these tables presented exactly the same information. Unfortunately, the actual source of fetal material used to create the 1939 table is also unclear and ambiguous. In 1938 Schour and Kronfeld applied 'tooth ring analysis' to one individual using the apposition rate from the 1937 work of Schour and Massler, (Schour and Kronfeld 1938:483) which presented the enamel apposition rate as ranging from 5-8μm for the central incisor (Schour and Massler 1937:350) rather than the 4μm daily rate that they cited elsewhere in their 1938 work (Schour and Kronfeld 1938:473). In 1939, the sample including the individual from 1938, consisted of two more infants and possibly an additional sample of '*approximately fifty complete jaws of infants and young children*' as well as the '*shed or extracted deciduous teeth of more than 600 additional children*' (Kronfeld and Schour 1939:25), again the Schour and Massler 1937 paper is referred to, although no actual rates are given in this text (Kronfeld and Schour 1939:20). Although this additional sample is mentioned, there are no details regarding whether or not these additional specimens were used to create the 1939 '*Chronology Of Human Deciduous Teeth*' table and there are no further details regarding which specimens were used to generate each section of this table.

The other values presented in the 1940 table are the '*Amount Of Crown Formed At Birth*' (again determined by the position of the neonatal line) and the '*Crown*

Completed Age' (Schour and Massler 1940a:1920). These values, although merged together do match the 1939 table and it is possible that these sections of the table could have been produced using Logan and Kronfeld's 1933 sample, as this sample included three specimens of newborns and 13 other specimens aged from two weeks to one year. The other probable sources of material used for this part of the table could be the briefly mentioned sample of 50 jaws of infants and young children, although it is possible that these were also the same specimens used in Kronfeld's 1933-1935c studies; as well as the loose teeth from more than 600 children and the three case studies of the children with birth injuries discussed above and cited by Kronfeld and Schour in 1939, although again this is unclear. In 1941, Massler, Schour and Poncher republished the 1940 diagram illustrating crown formation and completion times, no changes were made to these illustrated times.

It therefore appears that after tracking down the origins of this table which is still cited (Allan 1959; Lunt and Law 1974; McCall and Wald 1940) that the source of the material used to create it is unclear and ambiguous in origin. In addition to the pathological nature of part of this sample, which undoubtedly affected the resultant crown formation times, Logan and Kronfeld also stated that they '*do not consider the absolute ages*' of their '*specimens the most significant item*' (1933:393), which also brings the ages of their sample into question. So to conclude, it appears that the sample used to produce this table was probably of a very limited size, pathological and consisted of material of an uncertain age. This raises the all important question – how accurate could this often cited table actually be?

In addition to presenting their table, Schour and Massler (1940a) also reported the results of their observations to ascertain the number of growth centres from which enamel formation commences. Appositional growth centres were defined by Schour and Massler (1940a:1918) as the '*high point on the dentino-enamel junction or dentin cusp from which cellular activity begins at maximal velocity and radiates in a definite growth plan*'. Each of these growth centres supposedly gave rise to a '*lobe or cuspule*' in the anterior teeth and to a cusp in the

posterior teeth (1940a:1918). While the different tooth types '*differ only in the characteristic number and position of these growth centres*' (1940a:1918).

Although cellular differentiation of the enamel forming cells (ameloblasts) occurs before that of the dentine forming cells (odontoblasts), crown formation commences with the formation of a tiny cone shaped increment of dentine immediately under the growth centre (dentine cusp). The '*formation of enamel begins a few days later*' (Schour and Massler 1940a:1921). With the exception of this initial microscopic cuspal portion of dentine which precedes enamel formation, dentine and enamel apposition are synchronised so that for every layer of enamel that is apposed there is a corresponding layer of dentine in the crown. The incremental growth of each of these centres results in an increasingly larger cone shape, upon which, at daily intervals '*approximately 4µ*' (Schour and Massler 1940a:1919) of enamel are apposed one on top of the other by the ameloblasts. This 4µm daily rate continues until the full height of the cusp is reached. It is the functional life span of the ameloblast which limits the specific length of an enamel prism and which therefore determines the width or thickness of the resultant enamel, so when the life span of the ameloblast comes to an end the final width of enamel is therefore established (Massler and Schour 1946). Once the full height of the cusp has been reached, subsequent enamel layers are deposited only at the sides of the enamel cone in the form of concentric truncated cones; this continues until the full width of the crown and the enamel-cementum junction are reached and the crown is then complete. In the teeth consisting of multiple growth centres, the ameloblasts of the adjacent cones merge their cellular activity together so that the succeeding incremental layers coalesce and take on the form of the gross outline of the enamel-dentine junction. Schour and Massler (1940a) reported their histological and radiographic observations to ascertain the number of growth centres for each tooth type; incisors, they stated are formed from the coalescence of three growth centres lying mesiodistally, they suggested that the mamelons demarcated the position of these three growth centres. In the canines they suggested there were possibly three growth centres with the lingual cingulum being raised up into the beginning of a fourth growth centre. In the canine they suggested that the intermediate of the three growth centres lies above the

others, while in the incisors all three centres occupied approximately the same level. In the posterior teeth they stated that there was a separate growth centre for each cusp.

Although teeth begin their appositional growth at different ages, they do so in a '*regular and definite sequence*', (Schour and Massler 1940a:1924). According to Schour and Massler, apposition in the deciduous teeth commences with the central incisors and progresses posteriorly to the second molars. They also added that the time required for crown completion depends on the size of the crown and on the rate of apposition; for the deciduous dentition, the total time taken for crown formation is reported to be about seven to 14 months (Schour and Massler 1940a). Schour and Massler also stated that mineralisation occurs in the maxillary teeth slightly before it occurs in the mandibular teeth; although this is not expressed in their table, the diagram which they have presented does illustrate a very slight difference in the initiation and crown completion rates between the mandible and maxilla, with the central maxillary incisor initiating at four months in utero and the mandibular at four and a half months in utero.

Schour and Massler (1940a:1919) again reported the daily rate as being '*approximately 4μ*', however, no reference was made in this work to any specific range or deviation from this number, although this daily rate is reported as following the '*law of gradients*' (1940a:1921), which according to Schour and Massler is illustrated by the fact that '*cellular activity begins at maximal velocity*' (1940a:1918; 1940b:1793) and that increments nearest to the growth centre are farther apart than those increments nearer the enamel surface and anteroposterioly. This gradient was in addition to the two that were previously established by Schour in 1937, these being that there is a decreasing daily rate from the cusp to cervix (Schour and Massler 1937; Schour and Poncher 1937) and from anterior to posterior teeth (Schour and Massler 1937), (see **Section 8.1**). Additionally, no explanation was given as to why the 4-8μm range previously reported by Schour and Massler (1937:350) and the 5.5-8μm range reported by Schour and Kronfeld (1938:483) had been disregarded in this particular work, although these rates do reappear again in their 1946 work (Massler and Schour 1946:147). Interestingly Boyde (1963) cited a range of 2-

8µm in his age estimation paper, however no mention is made as to where these figures originated; although Massler and Schour are the only researchers mentioned in his work, the range that they proposed was actually 4.5-8µm (Massler and Schour 1946:147).

McCall and Wald (1940) published a copy of Kronfeld and Schour's 1939 table in their '*Clinical Dental Roentgenology*' text book. They added that even allowing for the discrepancy between radiographs and histological sections (see **Section 3.3**) their own observations of radiographs of '*a few fetal specimens*' (1940:97) indicated that there was considerable individual variation in the presented times and that there '*is not such a high degree of chronologic uniformity in tooth development during this period as has been assumed* (1940:97). Although McCall and Wald (1940:102) did not present any developmental data they stated that the upper incisors '*are occasionally more advanced than the lower incisors*'. However, in 1964, Boller suggested that the result of grouping several months together (e.g. four to five months) as McCall and Wald (1940) had done, had unfortunately caused them to miss '*many important*' details by '*failing to provide a representative sample for each fetal month*' (Boller 1964:78).

In 1959, Kraus criticised the work of Schour and Poncher (1937) stating '*there was no statistical analysis of the data, nor any attempt to assess the experimental and sampling errors*' (Kraus 1959a:134) and he stated that the rate of enamel apposition could not be a constant 4µm per twenty-four hours. To demonstrate this he dissected the tooth crypts of 76 fetal specimens, after they had been cleared and stained in situ with alizarin red S. The age of this fetal material ranged from 13 to 18 weeks in utero and was established by crown-rump measurements, although Kraus (1959a:140) stated that the use of crown-rump measurements '*is a crude indicator of age*'. Kraus measured the maximum mesiodistal diameter and the vertical thickness of the enamel over the cusp tips of maxillary and mandibular central incisors, maxillary lateral incisors and maxillary first molars (mesiobuccal cusp), as these teeth were '*consistently in some stage of calcification*' for the age range that these specimens covered (Kraus 1959a:136).

Statistical analysis demonstrated variable rates of enamel apposition both in the individual tooth and between different teeth; it indicated that the deciduous teeth do not mineralise at the same rate either mesiodistally or vertically and that the fastest rate of formation occurs in the mesiodistal dimension in all teeth. The maxillary central incisor was found to mineralise at a faster rate than the other teeth in both dimensions. Kraus (1959a:133) established that the '*absolute chronology*' of mesiodistal mineralisation had a sigmoidal growth curve, which indicated periods of alternating acceleration and deceleration of the mineralisation process. Kraus (1959a:144) concluded his work by suggesting that maybe '*genetic factors exert a* '*regional*' *control over the differential calcification rates*'.

In 1959, Kraus undertook another study in which he dissected the tooth crypts of 95 fetal specimens, after they had been cleared and stained in situ with alizarin red S. The age of this fetal material ranged from eight to 18 weeks in utero and again was determined by crown-rump measurements. It is not clear whether this sample included any of the specimens from his previous study which utilised specimens from 13 to 18 weeks. He again measured the maximum mesiodistal diameter.

Kraus (1959b:1130-31) stated that initial mineralisation in the deciduous dentition is extremely variable, in maxillary central incisors it '*may take place at any time from the twelfth through the sixteenth week*', with a mean age of 14 weeks. This figure of 12 weeks was derived from just three 13 week old specimens. Kraus (1959b:1131) added that '*the often-repeated assertion that this event takes place at four or four and a half lunar months is in error*'. For the second molars Kraus observed evidence of mineralisation as early as 14 (sample of one), 15 (sample of one) and 16 weeks (sample of two), again this is very different from the previously reported six months (Kraus 1959b). Unfortunately the age range of the sample prevented Kraus (1959b:1131) from observing the second molars in specimens over 18 weeks and from determining the age at which all the specimens exhibited initial mineralisation; although he stated that '*it seems doubtful, however, if this age would be greater than 22*

weeks or five and a half lunar months'. Kraus (1959b:1131) concluded that '*there is no fixed time of initial calcification for any particular tooth; rather each tooth has its own specific temporal span during which initial calcification may take place*'.

From his observations Kraus also suggested that the sequence of initial mineralisation should be amended to central incisor, first molar, lateral incisor, canine and second molar; rather than the regular progression from central incisor to second molar, which had previously been accepted. Evidently only one 15 week fetus did not follow this '*extremely rigid*' and '*definite sequence*' of initial mineralisation, in this case only the central incisor and one lateral incisor showed any evidence of mineralisation (1959b:1131 and 1136). In addition, the maxillary central and lateral incisors and the first molar commenced mineralisation before their mandibular counterparts, while the mandibular canine mineralised before the maxillary one and the maxillary and mandibular second molars began mineralisation simultaneously.

Kraus (1959b) disagreed with Schour and Massler (1940a) regarding the number of mineralisation centres that were present, he examined almost 200 central and 126 lateral incisors in various stages of mineralisation and concluded that there was not a single instance when more than one centre of mineralisation was observed, as opposed to the three that had been previously reported. Kraus (1959b:1134) stated that the '*mamelons cannot be vestiges of centres of ossification since there is only one centre of calcification in each deciduous incisor*'. Kraus (1959b:1134) concluded that mamelons '*simply mark the sites of depressions in the dentinoenamel junction which are roughly duplicated by the overlaying deposition of enamel*'. Kraus also observed 32 maxillary and 30 mandibular canines again in various states of mineralisation and again concluded that there was only one centre of mineralisation.

Turner in 1963 examined radiographs and stained serial sections from the jaws of 35 human fetuses ranging in age from eight to 40 weeks in utero, in order to determine the sequence of cuspal development in the molars. The ages of

Turners specimens were derived from crown-rump and crown-heel length measurements. Turner (1963:524) was aware that '*measurement alone is an unreliable method of estimating foetal age*' and he stated that '*whenever possible clinical data referring to the foetus should also be obtained*'.

Turner (1963:524) commented that the use of fetal material had the disadvantage of being '*based on material of a diverse origin*' and that this could result in '*genetic variations occurring which may not be detected during the investigation*'. Turner (1963:538) was also aware of the limiting factors of his '*small*' sample size and he added that it '*will be necessary to study further material to adduce these stages with greater accuracy*'.

During the early stages of development Turner identified that maxillary development was ahead of mandibular development; however he reported that in both jaws the first evidence of dentine formation was found at 18 weeks in utero in the first molar and at 19 to 20 weeks in the second molar. The order of cuspal development followed a '*definite sequence*' which Turner (1963:538) presented in the maxilla as being paracone, protocone, metacone and then the hypocone when this cusp is present. In the mandible the sequence was protoconid (mesiobuccal), metaconid, hypoconid, entoconid and hypoconulid. Turner also reported that '*dentine formation is always in advance of enamel matrix formation*' (1963:525).

Nomata in 1964 pointed out that previously three main methods had been used to study the chronology of the human deciduous dentition; these being dissection, radiology and histology and that unfortunately these methods had not produced results that were comparable with each other. Nomata presented a table detailing the times of the onset of mineralisation that had been collated from the work of previous authors and he included his own data for comparison. For the other authors cited in this thesis and who were also included in Nomata's table, it appears that he did not attempt to convert any of this data, (even months to weeks); unlike several more recent researchers, Nomata cited data directly from the original source (Lunt and Law 1974; Sunderland et al.

1987). Nomata (1964:71) concluded that the controversy between these different researchers was '*probably due to the difference in the number of materials investigated and the inconsistency of methods used*'.

Nomata studied the chronology of mineralisation of the deciduous teeth from 140 human fetuses aged using crown–rump length measurements. He used stained serial sections and dissection, it appears that magnification was not used with the dissection as '*naked-eye dissection*' is mentioned (1964:55).

The initial mineralisation times of both dentine and enamel were recorded and Nomata (1964:74) demonstrated that enamel formation occurs '*slightly after*' dentine formation in all tooth germs, unfortunately no exact times were presented. He further stated that the '*chronological difference between enamel and dentine formations is greater in the lateral incisors than in the central*' (1964:67). According to his text '*calcification of the upper central incisor commences at 114mm crown-rump length (17 weeks)*' (1964:71), however according to another table presented by Nomata (1964:70) the actual data that has been included in the comparative table is actually the time of the '*complete formation of dentine*' (1964:70).

Nomata (1964:71) agreed with Kraus that the sequence of mineralisation is '*quite rigid*' but he reported the sequence of mineralisation to be maxillary central incisor, mandibular central and lateral incisors, maxillary first molar and lateral incisor, mandibular canine and first molar, maxillary canine and second molar and finally mandibular second molar.

Nomata, like Kraus (1959b) did not find any evidence of more than one mineralisation centre in the incisors and he stated that mineralisation commenced at the middle mamelon and spread mesially as well as distally along the incisal edge. The order of cuspal mineralisation in the maxillary molars was found to be paracone, protocone, metacone and then hypocone. In the mandible the sequence was protoconid (mesiobuccal), metaconid, hypoconid, entoconid and hypoconulid, in other words the mesial cusps

mineralised earlier than the distal and the buccal cusps mineralised earlier than the lingual cusps.

Kraus and Jordan examined 787 aborted human fetuses using dissection and alizarin staining. This large sample size is ideal for such a study, unfortunately no details about the individual specimens were available and the nature of the abortion was unknown i.e. therapeutic or spontaneous. Although Kraus and Jordan (1965:27) only selected specimens '*exhibiting no obvious external malformations or signs of pathologic conditions*' for their sample, due to the lack of clinical records it is possible that some pathological or chromosomal anomalies may have been present. These specimens were aged using crown-rump or crown–heel length measurements and all of the specimens were preserved in 10% formalin.

Using magnification for the smaller specimens, the tooth follicles were dissected intact from their bony crypts and then stained with alizarin red S. The embryonic crown was then carefully dissected from the rest of the follicle under low magnification. The dissected crowns were drawn and photographed and the maximum mesiodistal and buccolingual dimensions were recorded.

Kraus and Jordan presented their results in '*Human Dentition Before Birth*', which was published in 1965. Kraus and Jordan (1965:119) stated that '*while rare exceptions may occur*' the sequence of initial mineralisation can be '*firmly established*' as central incisor, first molar, lateral incisor, canines, second molars (see **Section 3.5.2**). Kraus and Jordan also reported the sequence of cuspal mineralisation in the molars, which follows the same order as the previously reported cuspal sequences that have been described above (Nomata 1964; Turner 1963). Kraus and Jordan (1965:128) added that '*it is interesting to note the relatively close agreement among the three investigations*'. The complete sequence proposed by Kraus and Jordan can be found below (see **Section 3.5.2**).

Kraus and Jordan (1965:129) also discussed the observations raised previously by Kraus (1959a) regarding the presence of '*different rates of enamel apposition in different parts of the same tooth*'. Schour and Poncher (1937), Schour and Massler (1937) and Schour and Massler (1940a) had also previously suggested that differential appositional rates existed for different tooth types and also for different parts of the same tooth. Kraus and Jordan (1965:129) raised the main points of Kraus' 1959a study again, these being that in neither the mesiodistal or vertical dimensions does mineralisation occur at the same rate; that the maxillary central incisors appear to mineralise faster than the other teeth in both dimensions; that in any tooth cusp mineralisation proceeds faster mesiodistally than vertically; and finally that when the mesiodistal mineralised diameters of any tooth are plotted against time (age in weeks), a sigmoidal growth curve is produced. This resultant sigmoidal curve they suggested, indicated periods of alternating acceleration and deceleration of the mineralisation process, Kraus and Jordan (1965:131) added that this '*curvilinear regression*' is '*characteristic of postnatal skeletal growth*'.

Lunt and Law (1974) presented a very comprehensive literature review of the chronological studies of the deciduous dentition that had been previously undertaken. They presented a table of initial mineralisation times which they had collated from the work of previous authors, similar to Nomata (1964) had done, however Lunt and Law attempted to convert some of the original data. They stated that '*unless the results are converted to a common basis*' (1974:603) any comparison of the data presented by other authors was not possible and that conversion was required to enable such a comparison. This they felt was necessary as the age of the fetus can be calculated from two different points in time[3] (see **Section 3.1.2**). Lunt and Law (1974:604) stated that some of the values presented in their comparison table were '*different from those in authors original reports; they have been converted to fertilization age of the fetus, on the basis of authors' data on time of birth and fetal body lengths*'. Unfortunately the result of this conversion is confusing and for the authors that have been included above in this thesis this has resulted is the following:

[3] 'Menstrual age' is statistically two weeks earlier later than 'fertilization age'.

- Broomell and Fischelis (1913) has had two weeks consistently added to the original data, which seems reasonable as two weeks is the average time between ‘menstrual age’ and ‘fertilization age’.
- Meyer (1935) has had 1.5 to 2.5 weeks added to the original data.
- Kronfeld and Schour (1939) has remained the same as originally cited, as the method of ‘tooth ring analysis’ calculates backwards from birth, Lunt and Law (1974:604) have therefore assumed this method provided the ‘*true (fertilization) age of initial calcification*’.
- Turner (1963) has had two to three weeks added to the original data for the first and second molars.
- Nomata (1964) has had from three to 6.33 weeks added to the original data.
- Kraus and Jordan (1965) has remained unchanged from the original cited source, even though they used similar crown-rump length aging methods as Nomata (1964).

As a result of their literature review, Lunt and Law (1974:599) suggested that the table presented by ‘*Logan and Kronfeld, slightly modified by McCall and Schour*’ which has ‘*been an accepted standard since 1940*’ and which ‘*still appears in current textbooks*’ should be updated; although the data that they cited as being this table, is actually the table originally presented in 1939 by Kronfeld and Schour. Lunt and Law (1974:605) suggested that this chronology table should be modified, mainly due to the fact that the ages for initial mineralisation are ‘*based primarily on the method of tooth ring analysis, which is open to question*’. In addition the sample size used to develop this method was very small and some ‘*unproved assumptions were made about the apposition rates*’. Lunt and Law like Kraus (1959a) also criticised the lack of statistical methods and the failure to report error. They also pointed out that ‘tooth ring analysis’ was unable to indicate the ‘*correct sequence*’ of deciduous tooth mineralisation, which had since been ‘*established quite conclusively by more recent investigators*’ (1974:605).

Lunt and Law suggested that this chronology table should be updated to include the work of both Nomata (1964) and Kraus and Jordan (1965); as this more recent work was in close agreement as to the ages at which teeth initially mineralise and these values are '*based on the best evidence available*' (Lunt and Law 1974:605). Lunt and Law suggested that the mean ages and ranges of variation reported by Kraus and Jordan should replace those of Kronfeld and Schour. However, as Kraus and Jordan did not report any ranges for lateral incisors or canines, Lunt and Law obtained values for the early and late onset of mineralisation in these teeth from Nomata's work. For the mandible, however, only the lower end of the range for these teeth could be obtained from Nomata's data because the higher end was still earlier than the mean averages supplied by Kraus and Jordan. As a result of this update Lunt and Law suggested that the times of initial mineralisation of the deciduous teeth should be amended to be two to six weeks earlier than those that were presented in Kronfeld and Schour's (1939) original '*Chronology Of Human Deciduous Teeth*'.

As the result of these new initialisation times Lunt and Law (1974) also proposed that the sequence of mineralisation should now be updated to be central incisor, first molar, lateral incisor, canine then second molar; as presented by Kraus (1959b) and Kraus and Jordan (1965). These authors using large samples and methods independent of fetal age to derive this sequence had '*firmly established*' this new order (Lunt and Law 1974:604) which was also supported by the data provided by Nomata (1964). Lunt and Law (1974:606) stated that this new sequence '*is based on better evidence than the long accepted sequence*' which was the anterior to posterior progression from central incisor to second molar.

Lunt and Law (1974) concluded their suggestions with a table of amended initial mineralisation times, this presented the newly proposed earlier mineralisation times, with ranges alongside each average value. In this table they also amended the 'amount of enamel present at birth' for the molars, to correspond to the results of Kraus and Jordan, however they did retain the crown completion times from the original table by Kronfeld and Schour (1939).

As the evidence of Kraus (1959b), Kraus and Jordan (1965) and Nomata (1964) suggested that deciduous teeth mineralise in a range of times, rather than at fixed points in time as had previously been presented in the literature, Lunt and Law suggested that any future research should include ranges and mathematical means rather than the fixed values that had previously been presented, as these '*will better reflect the variations in development*' (1974:606). They also suggested that the division of differences according to jaws should be more specific as in general the '*maxillary teeth are usually ahead of the mandibular teeth in development*', with exceptions being the second molars, which generally are more advanced in the mandible and the lateral incisors and canines, which at times may also be more advanced in the mandible (Lunt and Law 1974:606).

Lunt and Law (1974:605) stated that the proposed modifications that they had suggested for the updated chronology were derived from results that have been '*based on measurements of fetuses to estimate age*'. They added that further research with fetuses with clinical histories was needed so that age may be more accurately determined. They concluded their review by stating that '*all methods of determining the time of initial calcification have been found to be imprecise because of the error inherent in fixing the age of the fetal specimens by measurement*' (1974:606). Although they then added that as their suggested sequence of mineralisation is not dependent on fetal age, it should be '*considered accurate*'. However any modifications to these updated initial mineralisation times Lunt and Law (1974:606) suggested '*should await the results of research using fetal specimens with accurate clinical histories for determination of age*'.

Using radiographs and dissections without the aid of microscopy, Boller (1964) compared the developmental stages of the deciduous tooth germs. The sample Boller used consisted of 200 fetal specimens aged from three to ten months, obtained by therapeutic and spontaneous abortions as well as stillbirths, some were fresh and some had been '*stored in formalin for varying periods of time*' (Boller 1964:69). The ages of the specimens were determined by crown-rump length measurements. Boller is the only researcher mentioned in this thesis to

have included any details about how he obtained his crown-rump lengths; he even supplemented his description with diagrams.

Boller exposed the dental follicles and opened the dental sacs of his specimens which were then cleared with potassium hydroxide and stained with alizarin red S. Radiographs were also obtained of the other half of the head. Unfortunately Boller only concentrated on describing the development the first molar, which he considered to be a '*typical tooth*' (1964:79). According to his observations the first molar showed radiographic evidence of mineralisation at the age of four months, his radiographs showed mineralisation of the mesiobuccal cusp '*as well as some calcification of the anterior teeth*' (1964:75). This observation was supported by his dissections and he presented both prints of his radiographs and photographs of his dissections to illustrate his findings.

Boller reported that his observations supported the work of Meyer (1935) and Schour and Massler (1940b and b) and that Gantz (1922) had also observed similar initial mineralisation at four months. He stated that this finding also supported the work of Kraus (1959b), who had reported initial mineralisation in maxillary central incisors at 12 to 16 weeks in utero. Boller reported that his observation of mandibular development slightly preceded that of maxillary development and that this supported the work of Meyer (1935) rather than Kraus (1959b).

The most recent complete chronological study that could be located in the literature occurred in 1987. Sunderland et al. (1987) attempted to establish initial mineralisation times by examining the maxillary and mandibular stained serial sections of 121 fetal jaws ranging from ten to 26 weeks 'gestational age' (post-menstrual age). Of their sample 64 were male, 55 female and two were of unrecorded sex,

From an initial sample of 184 fetal specimens, which had been stored in 10% formalin, 121 were selected, unlike previous studies Sunderland et al. (1987)

attempted to limit their sample to non-pathological material by excluding all specimens with gross microscopic abnormalities, chromosomal anomalies or inadequate medical histories. However, from their final sample, 100 of their specimens were the result of therapeutic abortion for 'social reasons' and 21 were the result of spontaneous abortion which are pathological by definition.

Unlike previous studies Sunderland et al. (1987) also tried to overcome the problem of accurately aging their specimens. They attempted to do this by only using specimens of known maternal history. The ages of their specimens were determined by several different methods including, maternal history, crown-rump length, crown-heel length, skull circumference, brain and body weight, the histological evaluation of cerebellar and renal development, as well as the assessment of gestational age by obstetricians, paediatricians and pathologists, resulting in an overall expert consensus for the age of each specimen. No details are presented about how this consensus was achieved, however this does appear to be the most accurately aged sample used to develop a dental chronology.

Using light microscopy Sunderland et al. (1987) examined each section from the ten and 12 week old fetuses as Logan and Kronfeld (1933) had done, however they then only examined every 15th section for the remaining specimens in their sample. They were looking for '*histological evidence of mineralised dentine*' (1987:168).

Sunderland et al. (1987) attempted to overcome another problem encountered by previous researchers, this being that previous researchers had not always been clear whether the times they presented for initial mineralisation referred to the first appearance of mineralisation in any tooth or the time when every tooth of that type in the sample exhibited mineralisation, or even whether a mean value was presented to indicate the date of initial mineralisation. In order to clarify this, Sunderland et al. (1987) presented their ages of initial mineralisation as a range over which initial mineralisation was observed in the dentine. This

included the youngest age at which mineralisation was first observed and the ages at which 50% and 100% of their specimens exhibited initial mineralisation.

An unfortunate problem experienced with the sample used by Sunderland et al. (1987:173) was that regrettably '*at the critical age of 19 weeks*' during which time teeth are undergoing or beginning to undergo mineralisation, there were only two specimens, as opposed to 13 specimens at week 18 and ten specimens at week 20. In addition both of the specimens at week 19 were female, which also prevented a comparison between sexes. According to the comparison table collated and modified by Sunderland et al. (1987:173) and included in their discussion, previous researchers had stated that initial mineralisation occurs at 19 weeks, therefore unfortunately by not having an adequate sample size for this time the resultant data are affected. A consequence of the small sample size of only two specimens from the 'critical' 19th week, is that as all data is recorded only as either 0%, 50% or 100%, this will decrease the degree of accuracy during the 19th week when compared to the other weeks. In all of the teeth examined by Sunderland et al. except for the second molar, at 19 weeks mineralisation is either occurring initially (canines), present in 50% of the sample (lateral incisors) or present in 100% of the sample (central incisors and first molars).

Unfortunately this missing data invites incorrect conclusions, for example, Sunderland et al. (1987) suggested that the entire sample of two central incisors show 100% initial mineralisation at 19 weeks, however, this is not a large enough sample size to confidently say this could not have occurred at 20 weeks. The lateral incisor, however, fortunately fits the general increasing trend of the graph presented by Sunderland et al. (1987:169) although only when the male and female figures are combined. The initial mineralisation of the canine may be first observed at 19 weeks; however, there is insufficient data to confirm this as the data for 19 weeks is derived from only one female specimen out of a sample of 121. The first molar, is similar to the central incisor, suggesting that the sample of two specimens shows 100% initial mineralisation at 19 weeks. However, again a larger sample size could equally show that this is actually

later at 20 weeks. In the second molar, initial mineralisation has been observed by other authors cited by Sunderland et al. to occur at 19 weeks however the data is lacking for this age in this study. So unfortunately the times of initial mineralisation established by Sunderland et al. (1987) could be inaccurate based on the lack of data from the 19th week at such a critical period. However it appears that Sunderland et al. (1987:173) have recognised that this small sample could bias their results and they stated that a study of more specimens from this age would '*possibly lead to slight modification of our conclusions*'.

Sunderland et al. (1987) established the order of initial mineralisation as central incisors, first molar, lateral incisor, canine then second molar. Although they stated that there was no '*consistent*' difference between the maxilla and mandible (1987:174), they identified that initial mineralisation first occurs in the maxilla for the central incisor and first molar; this was also reported by Nomata and is cited by Sunderland et al. (1987:173). Initial mineralisation also occurs first in the maxilla for the lateral incisor as well, although the range for 100% completion is shorter in the mandible; however it must be stressed that there were only two specimens at this age. The canine first shows initial mineralisation in the mandible. While mineralisation occurs simultaneously in the maxilla and mandible for the second molar, with the mesiobuccal cusps being the first to exhibit mineralisation.

Sunderland et al. found that the recording of the first evidence of mineralisation in each individual tooth covered a period of five weeks (15-20 weeks), whereas the times by which every tooth of a particular type exhibited mineralisation covered a period of three weeks (19-22 weeks). They reported that the time taken from the first appearance of mineralisation in one tooth to the entire sample exhibiting mineralisation varied between five weeks for the lateral incisors to two weeks for the second molars. They also found that no teeth exhibited mineralisation before 15 weeks, but that it was present in all of the teeth of all fetuses aged 22 weeks or more.

Although this study used specimens of known sex (except two specimens), Sunderland et al. (1987:174), stated in their discussion that there was no '*consistent sex difference*', however, their data suggests that this was not the case and that there was a slight difference with initial mineralisation being first observed in central and lateral incisors in males, while the canines mineralise first in females. Mineralisation occurs simultaneously in males and females in the first molar but it was first in males in the second molar. The age range over which initiation of mineralisation occurred was also slightly shorter in females, for the central and lateral incisors and was similar in males and females in the canines. The first molars are also similar although this is difficult to demonstrate due to the missing data. In the second molars the range, like the incisors was shorter in females. Unfortunately the sample size is so small that it is difficult to really divide the sexes (for example in week 19 there are no male specimens). The data in **Table 3.3** are derived from Sunderland et al's. combined male and female sample. Both male and female fetuses were examined by Sunderland et al, including two of unknown sex; however, the proportion of male to female specimens, although generally more of the former, varied for each age group. Although the data was recorded separately for each sex, it was combined to produce a total, which then formed the basis of the tabulated results. However, if one age group should favour one sex more than another and if sex is an important attribute to the initial time of mineralisation, then the resultant data would be skewed. Again this is a disadvantage caused by a small sample group.

The sample used by Sunderland et al. (1987:167) curiously favoured the even weeks between weeks 10 and 22, with more specimens present on the even numbered weeks, no explanation is given for this by Sunderland et al. However, one possible explanation for this bias, apart from the use of the material in other research projects, could be that the methods used to estimate fetal age might have included a calculation that originally determined fetal age in months, rather than weeks. Depending on how the 'consensus' view of all the age estimation methods was performed, of which no explanation is given, it could be possible that a measurement graded in months, or half months, could skew the data to favour months and half months, which would then translate to alternate weeks.

If this is the case, then the data derived by Sunderland et al. could also be skewed.

Sunderland et al. (1987) do not describe how the final figures that they presented in their table were derived from their results. Assuming that they have taken a mean average of the maxilla and mandibular teeth as their final initial mineralisation times, this would however, obscure the differences between the jaws, for example, the maxillary central incisors were shown to mineralise a week before the mandibular ones.

Sunderland et al. (1987), like Lunt and Law (1974) and Nomata (1964) included a comparative table of previous researchers work as well as the results of their own investigations. In order to enable a comparison Sunderland et al. (1987:173) stated that '*where necessary, we converted ages to menstrual age to facilitate direct comparison*'. Menstrual age is usually defined as two weeks before fertilization occurs (see **Section 3.1.2**). Unfortunately there is no indication in their table where they thought that this conversion was necessary. However, presenting their data as menstrual age rather than fetal or gestational age as other researchers have done, has resulted in confusion and the table of initial mineralisation dates derived by other researchers presented by Sunderland et al. (1987:173) is incorrect in several places. This is not only due to inconsistency in conversion but also due to inaccuracy, as it appears Sunderland et al. have also occasionally incorrectly cited data from the original studies.

For example, data from Peirce (1877), Peirce (1884), Broomell and Fischelis (1913), Kronfeld and Schour (1939) and Nomata (1964), have not had two weeks added correctly to convert data from in utero age to menstrual age.

- Peirce (1877) presented his original data as 'weeks', so assuming this refers to weeks in utero, according to Sunderland et al. this should mean that two weeks should be added to this data. However it appears that only one week has been added to the original data not two.

- Peirce (1884) presented his original data as ‘embryonic weeks’, so according to Sunderland et al. this should mean that two weeks should be added to Pierce’s 1884 data. This has not been done. In addition even allowing for the discrepancy between Peirce’s 1884 text and his table for the molars (18 or 19 weeks); the data cited by Sunderland et al. for the canines is incorrect, it should read 17 not the 19 weeks that they presented, (unless the two weeks has just been added to the canine data). So to conclude there appears to be both an error in citation and in conversion. Apart from the discrepancy between the text and the table in the 1884 data, this data is the same as that presented by Peirce in 1877 and it is reasonable to suggest that these two data sets should have been treated in the same manner.
- Broomell and Fischelis (1913) presented their original data as ‘fetal months’, so according to Sunderland et al. this should mean that two weeks should be added to this data. However, it appears that four weeks have been added to the incisors and canines and the data cited by Sunderland et al. for the molars is the same as the original source. So to conclude only a partial and incorrect conversion has occurred. Lunt and Law (1974) added two weeks consistently to the original data.
- Kronfeld and Schour (1939) presented their original data as ‘months in utero’, so according to Sunderland et al. this should mean that two weeks should be added to this data. This has not been done for the incisors which are separated by jaw; however it has been correctly done for the remaining teeth. So to conclude only a partial conversion has occurred. Lunt and Law (1974) did not change the original data.
- Nomata (1964) presented his original data as ‘weeks’, so assuming this refers to weeks in utero, according to Sunderland et al. this should mean that two weeks should be added to this data. This has not been done. In addition the data cited by Sunderland et al. for the maxillary canines is incorrect, it should read 21.33 not the 21.66 weeks that they presented. So to conclude there appears to be both an error in citation and in conversion. Lunt and Law (1974) added three to 6.33 weeks to the original data.

Even if the terminology has become confused or redefined over the years this still does not explain the partial conversations that seem to have occurred (Broomell and Fischelis 1913; Kronfeld and Schour 1939).

The data for Churchill (1932) have actually had two weeks subtracted, resulting in a four week discrepancy for the second molars.

- Churchill (1932) presented his original data as ‘interuterine months’ so according to Sunderland et al. this should mean that two weeks should be added to this data. This has not been done. In addition the data cited by Sunderland et al. for the second molar is incorrect, it should read 22 (same as the canine) not the 20 weeks that they presented, this is two weeks shorter than it should be. So to conclude there appears to be both an error in citation and in conversion.

Although the data for Kraus and Jordan (1965) have had two weeks added to the complete data set, they presented their data as ‘weeks’ similar to Peirce (1877) and Nomata (1964), which as mentioned above have been treated very differently, while Turner (1963) whose original data had been included with no conversion had also presented in ‘weeks’. Lunt and Law (1974) added two to three weeks to the original data for the first and second molars presented by Turner (1963) and although Kraus and Jordan (1965) had two weeks added by Sunderland et al., Lunt and Law (1974) did not alter this original cited data. Likewise Meyer (1935) presented his data as ‘months’ and no conversion has occurred on this occasion either, although Lunt and Law (1974) added 1.5 to 2.5 weeks to the original data.

Sunderland et al. have also incorrectly cited data, for example, Churchill’s (1932) data for canines and second molars are the same (22 weeks), yet Sunderland et al. have cited them differently (22 and 20 weeks respectively). Likewise Kronfeld and Schour (1939) for the mandibular central and lateral incisors the original source states 4.5 months, however these are cited differently as 18 and 20 weeks and Nomata’s 1964 data for maxillary canine is quoted as 21.66 weeks as opposed to 21⅓.

There are two resultant effects caused by these errors. Firstly the trend and sequence of initial formation in different teeth types, as determined by previous

studies, is incorrectly presented, for example teeth that develop simultaneously are quoted as developing weeks apart. The two data sets presented by Peirce (1877 and 1884) are the same in the original work (except for the text vs. table discrepancy), however only one data set has had a conversion of one week applied to it (1877).

Secondly, the specific timings are wrong, often by up to four weeks (Broomell and Fischelis 1913). Such errors are common and inconsistent throughout the table produced by Sunderland et al. resulting in the fact that any meaningful conclusion from the comparisons cannot be drawn.

Sunderland et al's. (1987) data appear to fall within a similar range to the other studies, however, when one considers that the other studies have been misquoted and shown to be up to four weeks more than they really are, Sunderland et al's. data seem much smaller (earlier) when compared to the other studies.

It addition to the confusion caused by the conversion to menstrual age, Sunderland et al. described how the gestational ages of their fetal sample were extensively confirmed by paediatric examination. They stated the age range as being 'post-menstrual ages' ranging from 10-26 weeks (1987:167). These ages are presented in a table but are labelled 'gestational age' rather than 'post-menstrual age'. Whether Sunderland et al. mean 'gestational age' to indicate 'fertilization age' and the necessary age conversion has not been applied in this table is unclear. Alternatively 'gestational age' may have been used to refer to age as a more general term, independent of its measurement method. The age and sample data for 'gestational age' and 'post-menstrual age' are identical, indicating that either the two are synonymous or the two week conversion described has not taken place. If the conversion has not taken place, the data

may be out by two weeks. Whichever definition was originally intended is unclear[4].

In order to prevent this error from occurring in this thesis, the data collated from the previous research described above consists of the original data taken from the original source, however, the reader is asked to bear in mind that there is an additional possible margin of two weeks due to the discrepancy caused in the aging of fetal specimens.

More recently Mahoney (2011) from a sample of 108 ground sections from an archaeological collection, presented the prenatal and postnatal crown formation times for deciduous molars, which he had obtained from observations of the incremental structures of enamel. The initial mineralisation times presented by Mahoney are later than those of Nomata (1964) and Kraus and Jordan (1965). Mahoney (2011:212) suggested that this could be due to the fact that these authors used staining techniques which would '*reveal the initial prism–free mineralised layer of enamel, and the time taken to form this layer would have been included in their values*'. The histological method used by Mahoney and also in this study, would not have accounted for this initial prism-less enamel as the prisms and cross-striations are not formed until the ameloblasts migrate away from the EDJ. Mahoney also added that as his sample was of an archaeological origin this may also have influenced his results, this is also a factor that may have influenced the results obtained in this study as teeth from the Spitalfields collection were included in the sample.

3.5 Discussion

The reports and studies reviewed above show considerable variation in initial mineralisation times for individual teeth. The data for the onset of mineralisation of the deciduous dentition found in the literature from the time of Jacobi in 1861

[4] In summary – 'menstrual age' is two weeks more than 'fertilization age'. To convert from fertilization to menstrual age, add two weeks. To convert from menstrual to fertilization age, subtract two weeks.

to the most recent publications show little consistency, varying from Robin and Magitot (1860-61) at the lower end of the range to Meyer (1935) at the upper end.

The data presented by the authors reviewed in **Section 3.4** has been collated and tabulated and can be found in **Table 3.3**. The data in this table has been cited directly from the original sources, the only conversions that have been applied are that data originally presented in ‘months’ or ‘days’ have been converted to ‘weeks’ (by multiplying by four, or dividing by seven). This is to enable an easier comparison of the results; however, in all cases the original data is present in parentheses. Although direct comparison of the ages at which deciduous teeth commence mineralisation presented by previous authors, may not be possible, unless the results are converted to a common basis, no attempt has been made to convert the data to ‘fertilization age’ as done by Lunt and Law (1974:604) or to ‘post-menstrual age’ as done by Sunderland et al. (1987:173). This is to avoid the confusion and inconsistency caused by the previous attempts to do this. However, the reader is reminded that there is a possible additional two week range due to the aging of the specimens used to produce this data.

The data in this table presupposes a normal expression of the inherent growth potential of the deciduous teeth; that is, that biological age of the individual is the same as chronological age (see **Section 3.1.1**). However, as discussed below interference in either the internal or external environment may cause wide variations in growth and development.

Table 3.3: Initial mineralisation table showing data collated from the literature review in chronological order, expressed in gestational weeks. If required conversion to weeks was performed, original data is in parentheses. Where information was available mandibular and maxillary teeth are presented separately.

Author	Date Of Publication	Form Of Original Data	Type Of Conversion	Central Incisor		Lateral Incisor		Canine		First Molar		Second Molar	
				Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular
Robin & Magitot	1860-63	Days	Days / 7	-	11.43-12.14 (80-85 days)	-	13.43-14.14 (94-99 days)	-	17.14-17.86 (120-125 days)	-	12.43-13.14 (87-92 days)	-	15.43-16.28 (108-114 days)
Peirce	1877	Weeks	None Required	17		17		17		18		18	
Legros & Magitot	1880	Weeks	None Required	16	16	16	16	16 in text. 17 in table.	16	17	17	17	17
Peirce	1884	Weeks	None Required	17		17		17		18 in chart. 19 in text.		18 in chart. 19 in text.	
Tomes	1889	Weeks	None Required	17		17		17		18		18	
Broomell & Fischelis	1913	Months	Months x 4	16 (4 mths)	16 (4 mths)	16 (4 mths)	16 (4 mths)	20 (5 mths)	20 (5 mths)	20 (5 mths)	20 (5 mths)	20-24 (5-6 mths)	20-24 (5-6 mths)
Tomes	1914	Weeks	None Required	20		20		24		24		24	
Mummery	1924	Weeks	None Required	20		20		24		24		24	
Brady	1924	Weeks	None Required	17		17		17		20		20	
Churchill	1932	Months	Months x 4	18 (4.5 mths)		18 (4.5 mths)		22 (5.5 mths)		20 (5 mths)		22 (5.5 mths)	
Wolfe	1935	Weeks	None Required	17 in text. 20 in chart.		17 in text. 20 in chart.		24		24		24	
Meyer	1935	Months	Months x 4	20 (5 mths)		20 (5 mths)		24 (6 mths)		20 (5 mths)		32 (8 mths)	
Kronfeld	1935c & 1937	Months	Months x 4	20 (5 mths)		20 (5 mths)		24 (6 mths)		20 (5 mths)		24 (6 mths)	
Schour & Kronfeld	1938	Months	Months x 4	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		22 (5.5 mths)	
Kronfeld & Schour	1939	Months	Months x 4	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		24 (6 mths)	
Schour & Massler	1940a	Months	Months x 4	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		24 (6 mths)	
Kraus	1959b	Weeks	None Required	12-16		-		-		-		14-22	
Turner	1963	Weeks	None Required	-		-		-		18		19-20	
Nomata	1964	Weeks	None Required	17	17.66 (17⅔)	19.66 (19⅔)	17.66 (17⅔)	21.33 (21⅓)	19.66 (19⅔)	19.33 (19⅓)	19.66 (19⅔)	21.33 (21⅓)	23.66 (23⅔)
Kraus & Jordan	1965	Weeks	None Required	14		16		17		15.5		19	18
Lunt & Law (average)	1974	Weeks	None Required	14		16		17		15.5 (15½)		19	18
Lunt & Law (range)	1974	Weeks	None Required	13-16		14.66-16.5 (14⅔-16½)	14.66 (14⅔)-	15-18	16-	14.5 (14½)-17		16-23.5 (23½)	17-19.5 (19½)
Sunderland et al.	1987	Weeks	None Required	15-19		16-21		19-22		16-19		20-22	
Mahoney	2011	Days	Days / 7	-		-		-		-	19-26	-	25-31

3.5.1 Timing of Initial Mineralisation

From **Table 3.3** it can be clearly seen that there is a very large range between the data produced by different authors and between each tooth type. In order to illustrate this more clearly the minimum and maximum data range for each tooth type is presented in **Table 3.4.a** below.

Table 3.4.a: Range of data collated for each tooth type (weeks gestation).

Central Incisor	Lateral Incisor	Canine	First Molar	Second Molar
11.43 - 20	13.43 - 21	15 - 24	12.43 - 26	14 - 32

However, as the lowest set of data was mainly produced by Robin and Magitot (1860-63) and as in their work the '*portion devoted to the origin and formation of the dental follicle was in many respects incomplete, and in some particulars erroneous*' according to Legros and Magitot (1880:3), the table below (**Table 3.4.b**) presents the same data without the work of Robin and Magitot (1860-63).

Table 3.4.b: Range of data collated for each tooth type (weeks gestation). Without the work of Robin and Magitot (1860-63).

Central Incisor	Lateral Incisor	Canine	First Molar	Second Molar
12 - 20	14.66 - 21	15 - 24	14.5 - 26	14 - 32

Although there is still a wide range for each tooth type, this range is not as pronounced as it was when it included the 'erroneous data'.

As suggested above in **Section 3.3**, the variation in mineralisation times that is illustrated in **Table 3.3** may have been due to the different methods and techniques used to establish the initial mineralisation and crown completion times by different authors. These methods and their advantages and disadvantages have been discussed above. Unfortunately not all of the studies reviewed in this work described the methods that were used to obtain data; however, where this information was available the initial mineralisation table was further divided up into these different methods (see **Table 3.5**). From this division there does not appear to be any major difference between each of the

Table 3.5: Initial mineralisation table showing data collated from the literature review in chronological order, expressed in gestational weeks. The data has been separated into the methods used to produce it and where information was available the sample size has been presented. Original data is in parentheses. Where information was available mandibular and maxillary teeth are presented separately.

Author	Date Of Publication	Methods Used	Sample Size	Central Incisor		Lateral Incisor		Canine		First Molar		Second Molar	
				Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular
Robin & Magitot	1860-63	Dissection and histology	No information provided	-	11.43-12.14 (80-85 days)	-	13.43-14.14 (94-99 days)	-	17.14-17.86 (120-125 days)	-	12.43-13.14 (87-92 days)	-	15.43-16.28 (108-114 days)
Legros & Magitot	1880	Dissection and histology	*'a large number'* (147)	16	16	16	16	16 in text. 17 in table.	16	17	17	17	17
Broomell & Fischelis	1913	Dissection and microscopy	*'about one hundred'* (ix)	16 (4 mths)	16 (4 mths)	16 (4 mths)	16 (4 mths)	20 (5 mths)	20 (5 mths)	20 (5 mths)	20 (5 mths)	20-24 (5-6 mths)	20-24 (5-6 mths)
Meyer	1935	Dissection and histology (serial sections)	12	20 (5 mths)		20 (5 mths)		24 (6 mths)		20 (5 mths)		32 (8 mths)	
Kronfeld	1935c & 1937	Radiography and histology (serial sections)	~30 from birth to 15 years (however probably only 1 for each of these ages)	20 (5 mths)		20 (5 mths)		24 (6 mths)		20 (5 mths)		24 (6 mths)	
Turner	1963	Radiography and histology (serial sections)	35 (8-40 weeks)	-		-		-		18		19-20	
Nomata	1964	Radiography and histology (serial sections)	140	17	17.66 (17⅔)	19.66 (19⅔)	17.66 (17⅔)	21.33 (21⅓)	19.66 (19⅔)	19.33 (19⅓)	19.66 (19⅔)	21.33 (21⅓)	23.66 (23⅔)
Sunderland et al.	1987	Serial sections	121 (10-26 post-menstrual age)	15 -19		16-21		19-22		16-19		20-22	
Schour & Kronfeld	1938	Tooth ring analysis	1	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		22 (5.5 mths)	
Kronfeld & Schour	1939	Tooth ring analysis	Uncertain	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		24 (6 mths)	
Schour & Massler	1940a	Tooth ring analysis (diagram)	Uncertain	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		24 (6 mths)	
Mahoney	2011	Histology (incremental counts)	108	-		-		-		-	19-26	-	25-29
Kraus	1959b	Dissection and alizarin staining	95 (8-18 weeks)	12-16		-		-		-		14-22	
Kraus & Jordan	1965	Dissection and alizarin staining	787	14		16		17		15.5		19	18
Peirce	1877	Literature survey produced a table	No information provided	17		17		17		18		18	
Peirce	1884	Literature survey produced a chart	No information provided	17		17		17		18 in chart. 19 in text.		18 in chart. 19 in text.	
Tomes	1889	Adapted from Magitot 1874	No information provided	17		17		17		18		18	
Tomes	1914	Adapted from Röse	No information provided	20		20		24		24		24	
Mummery	1924	Adapted from Röse and Tomes	No information provided	20		20		24		24		24	
Schour & Massler	1940a	Modified from Logan & Kronfeld 1933	25 (birth-15 years)	16-18 (4-4.5 mths)		18 (4.5 mths)		20 (5 mths)		20 (5 mths)		24 (6 mths)	
Lunt & Law (average)	1974	Literature review	None	14		16		17		15.5 (15½)		19	18
Lunt & Law (range)	1974	Literature review	None	13-16		14.66-16.5 (14⅔-16½)	14.66 (14⅔-)	15-18	16-	14.5 (14½)-17		16-23.5 (23½)	17-19.5 (19½)
Brady	1924	No information provided	No information provided	17		17		17		20		20	
Churchill	1932	No information provided	No information provided	18 (4.5 mths)		18 (4.5 mths)		22 (5.5 mths)		20 (5 mths)		22 (5.5 mths)	
Wolfe	1935	Possibly hypoplastic development	No information provided	17 in text. 20 in chart.		17 in text. 20 in chart.		24		24		24	

methods used. Although, the alizarin staining methods (Kraus 1959b; Kraus and Jordan 1965) do appear to produce slightly earlier results; as Mahoney (2011:212) stated above, this could be due to the fact that staining techniques '*reveal the initial prism–free mineralised layer of enamel, and the time taken to form this layer would have been included*' in the resultant data, in addition this layer may not be completely visible in radiographs or in histological sections. Lunt and Law (1974:604) stated that from their literature review it appeared that '*dissection methods generally did not yield ages for initial calcification at earlier times than did histological procedures*'.

Schour and Massler's (1940a:1921) suggestion that there is a two month difference between histological and radiographic techniques was not confirmed by this review. However it is possible that this may be due to the fact that several authors used multiple methods in their research, for example, Kronfeld (1935c:19) used serial sections which were '*fully substantiated by recent radiographs*'.

So to conclude, the statements made by the authors in **Section 3.3** above, describing how dissection methods provided earlier dates than radiography and how histology provided more accurate times than dissection, cannot be substantiated by this work. However, Mahoney (2011:211) directly compared several publications using radiographic methods not reviewed in this study, with his own histological observations and he concluded that his '*postnatal total crown formation times are greater compared to the mean postnatal age*' of molar crown completion times '*reported by the majority of radiographic studies*'.

The times for crown completion and the proportion of enamel present at birth have also been collated and tabulated and can be found in **Tables 3.6** and **3.7**.

Table 3.6: Crown completion table showing data collated from the literature review in chronological order expressed in weeks after birth. Conversion to weeks was performed, original data is in parentheses. Where information was available mandibular and maxillary teeth are presented separately.

Author	Date Of Publication	Form Of Original Data	Type Of Conversion	Central Incisor		Lateral Incisor		Canine		First Molar		Second Molar	
				Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular
Broomell & Fischelis	1913	Months	Months x 4	8 (2 mths)		8 (2 mths)		8 (2 mths)		8 (2 mths)		8 (2 mths)	
Meyer	1935	Months	Months x 4	12 (3 mths)		12 (3 mths)		48 (1 year)		36 (9 mths)		-	
Kronfeld	1935c & 1937	Months	Months x 4	16 (4 mths)		20 (5 mths)		36 (9 mths)		24 (6 mths)		40-48 (10-12 mths)	
Kronfeld & Schour	1939	Months	Months x 4	6 (1.5 mths)	10 (2.5 mths)	10 (2.5 mths)	12 (3 mths)	36 (9 mths)	36 (9 mths)	24 (6 mths)	22 (5.5 mths)	44 (11 mths)	40 (10 mths)
Schour & Massler	1940a	Months	Months x 4	6 (1.5 mths)	10 (2.5 mths)	10 (2.5 mths)	12 (3 mths)	36 (9 mths)	36 (9 mths)	24 (6 mths)	22 (5.5 mths)	44 (11 mths)	40 (10 mths)
Lunt & Law	1974	Months	Months x 4	6 (1.5 mths)	10 (2.5 mths)	10 (2.5 mths)	12 (3 mths)	36 (9 mths)	36 (9 mths)	24 (6 mths)	22 (5.5 mths)	44 (11 mths)	40 (10 mths)
Mahoney	2011	Days	Days / 7	-		-		-		-	39	-	56

Table 3.7: Proportion of crown completed at birth. Derived from data collated from the literature review in chronological order expressed as percentage of completion, where required conversion to percentage was performed, original data is in parentheses. Where information was available mandibular and maxillary teeth are presented separately.

Author	Date Of Publication	Central Incisor		Lateral Incisor		Canine		First Molar		Second Molar	
		Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular
Peirce	1884	'quite complete'		'quite complete'		66.66% (2/3)		66.66% (2/3)		50% (1/2)	
Mummery	1924	'crowns calcified'		'crowns calcified'		'tips calcified'		'cusps united'		'cusps united'	
Churchill	1932	50% (1/2)		40% (2/5)		25% (1/4)		'cusps united'		20% (1/5)	
Hess et al.	1932	66.66% (2/3)		66.66% (2/3)		25% (1/4)		'little more than the occlusal surface'		'base of the cusps' 'incompletely calcified'	
Meyer	1935	'almost completed'		'half complete'		-		-		'crown tips have coalesced'	
Kronfeld & Schour	1939	83.33% (5/6)	60% (3/5)	66.66% (2/3)	60% (3/5)	33.33% (1/3)		'cusps united'		'cusp tips still isolated'	
Schour & Massler	1940a	83.33% (5/6)	60% (3/5)	66.66% (2/3)	60% (3/5)	33.33% (1/3)		'cusps united'		'cusp tips still isolated'	
Lunt & Law	1974	83.33% (5/6)	60% (3/5)	66.66% (2/3)	60% (3/5)	33.33% (1/3)		'cusps united; occlusal completely calcified plus half to three fourths crown height'	'cusps united; occlusal completely calcified'	'cusps united; occlusal incompletely calcified; calcified tissue covers a fifth to a fouth crown height'	'cusps united; occlusal incompletely calcified'
Mahoney	2011	-		-		-		-	29%	-	19%

3.5.2 Sequence of Initial Mineralisation

Although most researchers agree that the central incisor is the first tooth to exhibit initial mineralisation and the second molar is the last, there is considerable disagreement over the order in which the other teeth mineralise. This non consensus of views is illustrated in **Table 3.8** where the times of initial mineralisation have been replaced by numbers indicating the sequence of initiation. Where identical initial mineralisation times have been provided these have been allocated the same sequence number.

As mentioned above, Jacobi (1861:402) stated that the order of dental development '*depends on the general rule of solidification in the foetal body, which begins in the median line and progresses to either side simultaneously*', however, in his next sentence he writes '*thus, the inner incisors are formed first, and the posterior molar teeth are formed last, with the exception of the canine, which appears later*'. As can be seen in **Tables 3.7** and **3.8**, this anterior to posterior sequence was perpetuated with very few variations. Schour and Massler (1940a:1924), stated that although teeth begin their appositional growth at different ages, they do so in a '*regular and definite sequence*'. According to Schour and Massler apposition in the deciduous teeth commenced with the central incisors and progressed posteriorly to the second molars.

From his observations in 1959, Kraus (1959b:1136 and 1131) suggested that the order of initial mineralisation was an '*extremely rigid*' and '*definite sequence*' and should be amended from the regular anterior to posterior progression, which had previously been accepted, to the sequence of central incisor, first molar, lateral incisor, canine and second molar. Evidently only one 15 week fetus did not follow this sequence and in this case only the central incisor and one lateral incisor showed any evidence of mineralisation. In addition, Kraus stated that the maxillary central and lateral incisors and first molar commenced mineralisation before their mandibular counterparts, while the mandibular canine mineralised before the maxillary one and the maxillary and mandibular second molars began mineralisation simultaneously.

Table 3.8: Initial mineralisation table showing data collated from the literature review in chronological order expressed in sequence. Where information was available mandibular and maxillary teeth are presented separately.

Author	Date Of Publication	Central Incisor		Lateral Incisor		Canine		First Molar		Second Molar	
		Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular
Robin & Magitot	1860-63	-	1st	-	3rd	-	5th	-	2nd	-	4th
Peirce	1877	1st		1st		1st		2nd		2nd	
Legros & Magitot	1880	1st	1st	1st	1st	1st	1st	2nd	2nd	2nd	2nd
Peirce	1884	1st		1st		1st		2nd		2nd	
Tomes	1889	1st		1st		1st		2nd		2nd	
Broomell &	1913	1st	1st	1st	1st	2nd	2nd	2nd	2nd	3rd	3rd
Tomes	1914	1st		1st		2nd		2nd		2nd	
Mummery	1924	1st		1st		2nd		2nd		2nd	
Brady	1924	1st		1st		1st		2nd		2nd	
Churchill	1932	1st		1st		3rd		2nd		3rd	
Wolfe	1935	1st		1st		2nd		2nd		2nd	
Meyer	1935	1st		1st		2nd		1st		3rd	
Kronfeld	1935c & 1937	1st		1st		2nd		1st		2nd	
Schour & Kronfeld	1938	1st	2nd	2nd		3rd		3rd		4th	
Kronfeld & Schour	1939	1st	2nd	2nd		3rd		3rd		4th	
Schour & Massler	1940a	1st	2nd	2nd		3rd		3rd		4th	
Kraus	1959b	1st		-		-		-		2nd	
Turner	1963	-		-		-		1st		2nd	
Nomata	1964	1st	2nd	4th	2nd	5th	4th	3rd	4th	5th	6th
Kraus & Jordan	1965	1st		3rd		4th		2nd		6th	5th
Lunt & Law (average)	1974	1st		3rd		4th		2nd		6th	5th
Lunt & Law (range)	1974	1st		3rd	3rd	4th	5th	2nd		5th	6th
Sunderland et al.	1987	1st		2nd		3rd		2nd		4th	
Mahoney	2011	-		-		-		1st		2nd	

Although Nomata (1964:71) agreed with Kraus (1959b) that the sequence of mineralisation was '*quite rigid*', he disagreed with Kraus about the actual sequence, he stated that the mandibular lateral incisor preceded the maxillary lateral incisor. Nomata reported the sequence of mineralisation to be maxillary central incisor, mandibular central and lateral incisors, maxillary first molar and lateral incisor, mandibular canine and first molar, maxillary canine and second molar, mandibular second molar. Kraus and Jordan (1965:119) argued that Nomata's sequence was not supported by other data that he had presented in his own work and that '*Nomata's various tables of calcification not only does not confirm this sequence but in fact refutes it*'.

Kraus and Jordan (1965:119) concluded that '*while rare exceptions may occur, the sequence can be firmly established*' as central incisor, first molar, lateral incisor, canines, second molars. Calonius et al. (1970:873) were able '*to confirm the chronology of tooth calcification*' presented by Kraus and Jordan, based on their observations of serial sections from 92 specimens aged from seven weeks in utero to three years. In addition from the conclusions made from their extensive literature review, Lunt and Law (1974:606) also supported this amended sequence of mineralisation. They stated that this new sequence '*is based on better evidence than the long accepted sequence*' which was the linear progression from central incisor to second molar. Lunt and Law (1974:606) also stated that as this sequence of mineralisation was not dependent on fetal age, it should be '*considered accurate*'.

It is agreed in the literature that the mesiobuccal cusp (protoconid/paracone) is generally the first to mineralise but again there is much debate regarding the other cusps. Enamel growth commences in the mesial cusps and then progresses to the distal cusps (Mahoney 2011). As this thesis is not concerned with cuspal sequence further than establishing that the mesiobuccal cusp is the first to exhibit initial mineralisation, this area will not be discussed in detail. However, the general consensus seems to support Kraus and Jordan (1965:122) and is as follows:

1) Protoconid and paracone of first molars.
2) Protoconid and paracone of second molars.

3) Metaconid and protocone of first molars.
4) Metacone of first molars.
5) Metaconid and protocone of second molars.
6) Metacone and hypocone of second molars.
7) Hypoconid of first molar.
8) Entoconid of second molars.
9) Hypoconulids of first and second molars.
10) Hypocone of second molars.

In general mandibular development has been reported as being ahead of maxillary development, Jacobi (1861:402) stated that this was '*in correspondence with the earlier ossification of the lower jaw in foetal life*'. Robin and Magitot (1861), Broomell and Fischelis (1913), Churchill (1932), Meyer (1935), Logan and Kronfeld (1933) and Boller (1964) all observed initial mineralisation first in the mandible while Gantz (1922) stated that it was about the same in both jaws. However Schour and Massler (1940a), McCall and Wald (1940), Turner (1963) and Lunt and Law (1974) all disagreed and claimed that the maxilla was first to exhibit initial mineralisation.

3.6 Limitations in Determining Deciduous Crown Chronologies

Several factors could have influenced the wide spread of the data presented in **Table 3.3**. These include the actual material being examined and the methods and techniques used to examine it. The methods have already been described above (see **Section 3.3** and **3.5.1**) and will not be discussed again; while the material and techniques used to obtain the data are discussed below.

3.6.1 Material Used in Determining Deciduous Crown Chronologies

One main limitation of the material used in the studies reviewed, (except for that of Sunderland et al. (1987:167) who used many different methods of age

estimation and then arrived at a '*consensus view*'), is that the age of the material was often determined by standard tables of body measurements, for example the crown-rump length, which as Sunderland et al. (1987:167) pointed out '*gives only an indirect estimation of gestation*'. Lunt and Law (1974:606) concluded their literature review by stating that '*all methods of determining the time of initial calcification have been found to be imprecise because of the error inherent in fixing the age of the fetal specimens by measurement*'. They also stressed that further modifications of the times of initial mineralisation '*should await the results of research using fetal specimens with accurate clinical histories for determination of age*'. Likewise Turner (1963:524) was also aware that '*measurement alone is an unreliable method of establishing foetal age*' and he suggested that whenever possible clinical data referring to the material used should be obtained. Several factors are responsible for the '*error inherent in fixing the age of the fetal specimens*' (Lunt and Law 1974:606) and these will now be discussed.

3.6.1.a Individual Variation in Human Development

The age estimation of an individual involves first establishing a biological age and then attempting to correlate it with a chronological age. In order to do this the specimen being aged must be compared to a 'known standard'; unfortunately as a result of this process incompatibilities are inevitably introduced. In addition, normal human populations show considerable variation in fetal development and there are many influences, extrinsic and intrinsic that can affect this development (Roberts 1976). The material that has been used in previous studies has mainly come from fetal material obtained from spontaneous or elective abortions and while the latter may technically be considered to constitute a normal sample, the former may have exhibited pathological abnormalities that would negate the usefulness of the resultant data (see **Section 3.6.1.c**). In addition, even if completely non-pathological fetal material is studied, a number of factors including individual variation, sex, race, genetic growth potential of the fetus, placental function and length of gestation may all influence fetal development; as well as maternal age, height, weight, state of nutrition, parity of the mother, single or multiple occupation of the

uterus, previous termination of pregnancies and the introduction of teratogenic components such as alcohol, nicotine and other drugs. Fetal development is also affected by the socioeconomic and ethnic group of the mother as well as being population-dependant. Even the external environment in which the mother lives, for example, altitude, season or climate could affect development (Roberts 1976). Each one of these factors could influence fetal growth and development and this may in turn affect the results obtained, unfortunately in most cases such information would probably have been unknown or unavailable to the previous researchers.

3.6.1.b Establishing an Age for the Source Material

As well as the wide variation in development, babies are also born at different gestational ages and therefore at different stages of development (Roberts 1976) and the terms ‘at birth’, ‘term fetus’ or ‘newborn’ are only of relative value in determining the degree of development (Kronfeld 1935a; Kronfeld 1935b; Logan and Kronfeld 1933). There are marked individual variations between different newborn infants, not only in body weight, but also in the degree of mineralisation of the bones, as well as in other respects (Kronfeld 1935b). Any attempt to lay down a ‘known standard’ for the dental structures of the newborn will therefore be futile unless exact data are available giving the birth weight, size and other somatic characteristics of the child from whom the dental specimens have been obtained. Unfortunately, this information in most of the studies reviewed was not available to the previous researchers. Kronfeld (1935b) stated that the best that he and judging from the literature others working along the same line had been able to do, was to obtain jaw specimens with the annotation ‘newborn infant’ or ‘term fetus’.

As mentioned above the direct comparison of the ages at which deciduous teeth commence mineralisation presented by other authors, may not be possible unless the results are converted to a common basis, or the reader is made aware of the possible variations that may exist in the data that is presented. Nomata (1964) compiled a similar table as has been presented here

(see **Table 3.3**) without any conversions, however, Lunt and Law (1974) and Sunderland et al. (1987) both attempted to convert the original data and as described above they did so with varying degrees of success. Conversion is possibly necessary because the age of the fetus may be calculated from two different points in time (see **Section 3.1.2**). However, both Lunt and Law (1974) and Sunderland et al. (1987) attempted this conversion and both came up with entirely different ages for the same original data.

The actual age of the original source material used to form the 'known standards' greatly influences the interpretation of the data collected. In previous studies of deciduous crown development, the age of the material studied was usually poorly documented and as mentioned above may have only been expressed in terms of the crown-rump length, crown-heel length or foot length. Although the ranges of weights and lengths of a baby at term are subject to variation the possible causes of which are listed above, for forensic purposes in the UK these measurements are generally taken as weight being 2550-3360g, crown-rump length (CRL) being 28-32cm, crown-heel length (CHL) being 48-52cm and head circumference being 33-38cm (Saukko and Knight 2004; Shepherd 2003). However, Saukko and Knight (2004:448) warn that '*morphological measurements are by no means infallible indicators of chronological age*'.

Use of CRL to Establish an Age for the Source Material

Unfortunately, due to the incomplete or inaccurate documentation of fetal material, researchers have often had to resort to deriving fetal age from manual measurements such as crown-rump length, crown-heel length or foot length of the fetus, or any combination of these (Churchill 1935; Gantz 1922; Kraus 1959a; Kraus 1959b; Kraus and Jordan 1965; Kronfeld 1937; Legros and Magitot 1880; Nomata 1964; Turner 1963). These measurements are then converted to a fetal age using other researchers published conversion data consisting of either, graphs, tables or equations. This procedure however, introduces more variation into establishing the actual age of the fetal material used to form the 'known standard' for later comparison (Bagnall et al. 1975;

Birkbeck 1976; Noback 1922; Scammon and Calkins 1923; Scammon and Calkins 1929; Streeter 1920).

The relationship between various manual measurements and the gestational age is discussed at length by the above authors however; the most common measurement used to estimate the fetal age is the crown-rump length, or sitting-height which involves the head, neck, trunk and pelvis. Wigglesworth (1996b:27) recommended that CRL should be routinely taken during a perinatal post-mortem examination and recorded to the nearest 0.5cm (and foot length measured to the nearest 1mm). However, more recently, obstetric ultrasound has been used to determine the most suitable manual measurements for aging formalin fixed human fetuses. Using an un-aged population Croft et al. (1999) compared established standard manual measurements of age estimation against the age estimated by obstetric ultrasound, they found that of the manual measurements both foot length and head circumference were superior to CRL. They found that manual CRL measurements were on average larger than those taken by ultrasound, while the manual foot length measurement tended to be smaller than those taken ultrasonically. Croft et al. (1999) used Streeter's (1920) research (as Sunderland et al. (1987) had done) to convert their manual measurements to fetal age; however the discrepancy between the two aging methods had the ultimate effect of producing two different fetal ages. The use of the manually estimated foot length gave a menstrual age to within two weeks of the reference standard (age determined by ultrasound), while the manually estimated CRL ages were inconsistent with the ultrasound with errors of up to six weeks in the older fetuses. This inaccuracy, however, may have been due to the fact that after the first trimester, distortion of the spine was evident resulting from compression due to inappropriate storage.

Unfortunately for aging purposes, it appears that the CRL is a rather inexact measurement and can vary considerably, in addition to the normal range of biological variation in the CRL, the degree of fetal flexion also has an effect on this measurement (Bagnall et al. 1975). Once removed from the uterus the fetal position is open to wide interpretation, a live fetus should be in a fully flexed position for CRL measurement by ultrasound, however, Croft et al. (1999:89)

found that it was not always possible to manipulate a formalin preserved specimen into a suitable position and therefore they concluded that the manual and ultrasonic CRL measurements of the older fetal specimens were not comparable, '*indeed, some values were meaningless*'.

Streeter (1920:148), who developed one of the most well known fetal age conversion tables, measured his smaller specimens '*without disturbing their natural curved posture*', similar to Croft et al. (1999), however, he straightened out the body of his larger specimens before recording the CRL and specimens that were extremely flexed and fixed so that they could not be straightened, he disregarded altogether. As a result of these differing postures, Streeter was unable to compare his older and younger specimens. Bagnall et al. (1975) measured the CRL of fetal specimens in the position in which they were initially fixed in formalin, they then compared these measurements with those from several published sources and found that the measurements varied considerably. They suggested that these discrepancies were caused by the lack of standardisation in the fetal posture when measuring the CRL. Indeed Bagnall et al. (1975), suggested that unless the fetal posture is standardised the accuracy of manual measurements of the CRL is questionable. Boller (1964) is the only researcher mentioned in this review to have included any details about how he obtained his CRL measurements; he further supplemented his description with diagrams.

Even inappropriate storage conditions of fetal material have an effect on CRL measurement. Croft et al. (1999:89) found that due to poor storage conditions, the natural spinal curvature of some of their larger specimens had become so distorted that although manual CRL measurements could still be made, they felt that these '*did not reflect the true CRL*'.

The CRL is just one measurement that can be used to determine fetal age, had multiple measurements been obtained, then this may have produced a more accurate age estimation; unfortunately this does not seem to be the case in the majority of the published research with just the CRL being used to determine fetal age, except in the case of Sunderland et al. (1987).

Affects of Fixation and Preservation on CRL

Embryonic length is the most common index used to determine fetal age and as discussed above it is highly variable. Early dental development studies were often performed on aborted material, virtually always fixed in formalin for varying periods of time (Gantz 1922; Kraus and Jordan 1965; Sunderland et al. 1987). Apart from possible errors in technique such as inaccuracies in specimen manipulation and orientation, reading the measuring equipment and possible clerical errors in recording the observations, there are also sources of error such as changes in the body form of the specimen before fixation. Artefacts produced by simple immersion fixation in formalin and artefacts produced by embalming and subsequent immersion preservation in formalin may also have an effect on the estimation of the fetal age using CRL. In addition formalin is also known to demineralise hard tissues when specimens are left immersed in it for long periods of time. Streeter (1920:48) stated that '*if it were not for the effect of the preservative*' there would probably be less variation in the CRL in his results. Birkbeck (1976:42) studied fresh unfixed fetal material and suggested that the differences between the work of Scammon and Calkins (1929) and of Noback (1922) were due to the '*differential effects of formalin fixation*' as well as '*slight flexion of the specimens during measurement*' the latter is discussed above.

This artefactual size change caused by formalin fixation and preservation may vary depending on the fixation method used, as well as the age, size, condition of the specimen and duration of preservation, furthermore this change may consist of either an increase or decrease in fetal size (Patten and Philpott 1921; Scammon and Calkins 1929; Schultz 1919; Streeter 1920; Tucker and O'Rahilly 1972). Several investigators have examined the effect of 10% formalin (4% formaldehyde) preservation on fetal specimens (Scammon and Calkins 1929; Schultz 1919; Streeter 1920). However, even before a fetal specimen is fixed, shrinkage by dehydration can occur, particularly in smaller fetal specimens, this source of error can be avoided by disregarding these specimens; however slight dehydration may not always be obvious.

Two methods of fixing fetal material are recorded in the literature and it appears that both have been encountered by researchers of dental development. The first method is to simply immerse the specimen in 10% formalin solution and the second is to embalm it, by injecting a 10% formalin solution into the umbilical artery (Streeter 1920) or vein (Scammon and Calkins 1929), tying off the cord and then immersing the specimen in a 10% formalin solution.

When fresh tissue is placed in formalin solution it undergoes considerable change both in size and weight. Young fresh fetal specimens when placed in 10% formalin solution quickly absorb the fluid becoming swollen and distended; they increase markedly in weight and length. In older fetal specimens and in those whose tissues are macerated, this distension is not so great. After this initial distension following continued storage in formalin, in the course of a few months the specimen tends to gradually regain its original size and weight. Streeter (1920) noted that although formalin distends the soft tissues in the initial stages of fixation, a fetus usually regains its normal proportions after 16 months of preservation. After increased periods of immersion in 10% formalin the fetus then starts to shrink (Scammon and Calkins 1929). This change of body size would have the effect of altering the CRL and therefore the resultant age of the specimen.

Although some authors reported using fresh material (Logan and Kronfeld 1933; Robin and Magitot 1863) most of the specimens used to develop dental chronologies appear to have been formalin fixed (Gantz 1922; Kraus and Jordan 1965; Sunderland et al. 1987). The affect of formalin fixation on fetal material, either via embalming or simple immersion has been shown to introduce an artificial element which results in the variation of the CRL over a varying period of time; this is another factor that must be taken into account when using CRL in the determination of fetal age. This is further complicated when fresh and fixed fetal specimens are included in the same study (Streeter 1920) and which Schultz stated is '*unsafe*' (1919:35). Boller (1964:69) reported using both fresh and formalin fixed specimens, some of which had been '*stored in formalin for varying periods of time*'. Gantz (1922) used specimens that had been embalmed via the umbilical vein as part of his sample, however, Streeter

(1920:148) had previously identified that by using this method of preservation the artificial increase in weight was so considerable that specimens preserved in this way '*cannot be fairly compared with those simply immersed in formalin*'.

None of the authors reviewed above however, seem to have taken the effects of the fixative and preservative solutions or the method of storage in to account when determining their CRLs.

3.6.1.c Affects of Pathology on the Source Material

The prenatal material that has been examined in order to determine initial mineralisation times had mainly been obtained from the result of spontaneous or elective abortions, as mentioned before while the latter may technically be considered to constitute a 'normal' sample; the former may have exhibited pathological abnormalities that would negate the usefulness of the resultant data. In addition Boller (1964) reported using a mixture of spontaneous and therapeutic abortions as well as stillbirths in his sample.

The size of the developing human body varies primarily with gestational age, however, the normal prenatal growth rate may be altered by the genetic or environmental factors which are discussed above, as well as being affected in characteristic ways by malformation syndromes and placental insufficiencies (Wigglesworth 1996b). Fetal growth can be modified by pathological interference (Roberts 1976) and most abnormal growth patterns in the perinatal period involve growth retardation rather than growth acceleration (Wigglesworth 1996a). As discussed above, data on fetal age is usually very limited and the CRL has often been used to determine fetal age, however throughout the perinatal period the CRL is profoundly influenced by pathological conditions affecting the growth potential and/or nutrition of the fetus (Wigglesworth 1996a). Unfortunately the criteria as to whether or not specimens are normal, like the age data, is also frequently unavailable and unreliable (Streeter 1920:145). As well as affecting the CRL there is no way of determining whether the dental development has also been affected by pathology.

In the previous studies several authors have knowingly included pathological material in their samples (Logan 1935; Logan and Kronfeld 1933; Schour and Kronfeld 1938; Schour and Poncher 1937). However as Sunderland et al. (1987:167) pointed out, pathological material '*does not usually conform to physiological norms*'. Other authors have attempted to remove pathological specimens from their sample (Kraus and Jordan 1965), although as already mentioned, these researchers have not always had access to complete clinical records and medical histories. So even if they had successfully managed to exclude all specimens with gross abnormalities, it is very likely that fetuses with minor chromosomal anomalies may not have been detected when these researchers selected their samples. Furthermore there is no way of determining if the pathological condition which had spontaneously terminated the pregnancy might also have interfered with the previous growth and development of the fetus. Although Sunderland et al. (1987) attempted to remove all pathological specimens from their fetal sample by excluding all specimens with gross microscopic abnormalities, chromosomal anomalies or inadequate medical histories, 21 of their specimens were the result of spontaneous abortion, which as mentioned before are pathological by definition.

Although the sample used by Logan and Kronfeld (1933) and Logan (1935) in their research was of a pathological origin, including infants who were stillborn, at term and others who died after prolonged illness or acute short term illnesses, Kronfeld (1935b:1138) defended the use of pathological material in his work. He stated that '*all science of human embryology, histology and anatomy, with very few exceptions (material obtained from surgical operations and from executions) is based on the same type of material*' and that this is the same type of material that he used in his research, i.e. from individuals who died of various diseases. However, Logan and Kronfeld (1933:394-395) concluded that '*there was by no means a constant ratio between the degree of calcification and the age of the child*' and they suggested that this may partly be '*due to the fact that in some of these children, development was retarded by prolonged illness*' and in other cases it may be the '*result of individual variations within a physiologic range*' (Logan and Kronfeld 1933:395). There are wide

variations in the development of the human body even in healthy children, and consequently more so in sick children who finally die of the disease that they had suffered from. Because of this wide variation in body development Logan and Kronfeld were more concerned with identifying the sequence of mineralisation rather than its exact timings, which they stated are naturally subject to wide individual variation and which are exaggerated in their case by the pathological nature of their sample.

In addition to the affect of pathology on the developing fetus it is impossible to estimate the precise time of death (Kellokumpu-Lehtinen 1984). Most cases of spontaneous abortion in the second trimester involve normally formed fetuses, however, many of them are often retained in utero for several weeks following fetal death (Wigglesworth 1996c). So it is possible that the development of the fetus may have ceased some time before its expulsion (Streeter 1920) or surgical removal and this may also effect any data obtained in relation to the age of the fetus.

In the UK it is possible to obtain a legal abortion for medical or social reasons up to 24 weeks. This has the affect of biasing the fetal samples studied, as more ‘normal’ material is available from this time period. As a result there are a larger number of fetuses of ages under 24 weeks in the majority of studies. Due to the fact that abortions are only legal until a certain date, the samples used by researchers are biased towards the younger ages and as a result the data from different sources is often merged to create a complete span of the fetal period, for example the work of Streeter (1920). Therefore introducing other sources of error and possible discrepancies, due to differing definitions as to what constitutes a normal specimen, the method used for taking measurements etc. which may further invalidate the results, maybe to such a degree that it would more than offset the advantage derived from using the larger sample size.

As a consequence of this bias in the sample caused by the time limit of legal abortion i.e. non-spontaneous (before 24 weeks), it therefore seems reasonable to suggest that post 24 weeks the material is obtained from spontaneous

abortions and is more likely to be pathological in nature. Although the number of current legal abortions has increased in the UK and the use of modern genetic screening can be used to eliminate pathological specimens from samples, combined with the opportunity of obtaining more accurate fetal ages with the use of modern ultrasound techniques (Rossavik and Fishburne 1989), all of which may significantly decrease the errors described above, it seems unlikely due to changes in the current legislation and in particular the Human Tissue Act 2004, that any research of this kind will be carried out in UK in the near future.

Age is an independent variable against which growth and development are always assessed. The major problem of using fetal material in growth and developmental research is that of determining the exact age of the original source material. Unfortunately determining fetal age cannot be done with any real accuracy as it depends upon either the mother's recollection of her menstrual dates or upon measurements of the fetus which are then converted to a derived fetal age, which as discussed above for numerous reasons can be extremely variable. With varying degrees, each of these factors may influence the determination of fetal age, however, despite these difficulties, researchers have had to establish the 'best estimate' of fetal age that they can and then use this derived age to generate and establish the 'known standards' against which other researchers can then compare their own fetal material in order to generate their own data. Unfortunately the possible sources of error encountered when using fetal material are not usually described in the dental literature and do not appear to have been taken in to account in many of the studies reviewed.

3.6.2 Techniques Used in Determining Deciduous Crown Chronologies

3.6.2.a Cross-sectional Studies

One main disadvantage of the examination of fetal specimens in the determination of crown formation times is that because of the nature of the material studied only a cross-sectional study can be achieved. In addition the

specimens used to study the prenatal development of the deciduous crown, are from an entirely different source of material than the specimens used to study postnatal crown development and this introduces another potential source of error, as the source material is from different groups of individuals. Kraus (1959a) raised the disadvantage of a cross-sectional study in his work and pointed out that each data point is represented by a different individual of a different age and is at a different stage of mineralisation. In the case of Sunderland et al. (1987) this has created some curious data, for example, for the male maxillary central incisors at week 17, 82% of the sample show initial mineralisation, while at week 18, 67% of the sample show initial mineralisation, which is inconsistent with our understanding of growth. Furthermore at 19 weeks 0% of initial mineralisation has occurred as there is no sample material for this age and this further confuses the matter.

Boller (1964) suggested that if several specimens are studied, radiographed and dissected for each lunar month, as he had done in his work, then '*regular incremental development month by month can be detected as it would be expected*' (1964:78). He also added that the '*occasional retardation or advancement of maturity detected between monthly stages can be explained as individual variation such as would occur in a cross-sectional study*' (1964:78).

3.6.2.b Sample Size

In the majority of cases discussed above the sample sizes have been very small, sometimes consisting of only a few individual specimens (Logan and Kronfeld 1933; Schour and Kronfeld 1938; Schour and Poncher 1937; Tomes 1914), (see **Table 3.5**). However, some of the more recent studies have used considerably larger sample sizes (Kraus and Jordan 1965; Mahoney 2011; Nomata 1964; Sunderland et al. 1987).

Although the sample used by Kronfeld (1935c) in the production of his deciduous chronology consisted of about 30 individuals and Kronfeld

(1935b:1139) stated that his work was '*not based upon the examination of one or two specimens, but on a systematic study of nearly thirty human jaws of different ages*', in the spread of this sample of 30 individuals over the age range required to produce the deciduous chronology, there was often only one specimen for each specific age.

Kraus (1959a and 1959b) criticised the work of other authors for their lack of statistical analysis however, in his own work he stated that the age of initial mineralisation of the second molar could be as early as 14 weeks, although this statement is based only on one specimen (Kraus 1959b). The effect of a small sample size was illustrated by Sunderland et al. (1987:173) who unfortunately only had a sample of two specimens '*at the critical age of 19 weeks*'. In addition both of these 19 week specimens were female, which also prevented a comparison between sexes. Turner (1963:538) was also aware of the limiting factors of his '*small*' sample size and stated that it '*will be necessary to study further material to adduce these stages with greater accuracy*'.

However, as discussed above it is very unlikely that the fetal material available for such studies will increase in the near future and researchers will either have to re-examine previous material or find new ways to investigate initial mineralisation.

3.6.2.c What Is Actually Being Measured and Reported?

It is not always clear in the literature whether the data presented in dental chronology tables for initial mineralisation refer to the first evidence of initial mineralisation in any tooth or the time when every tooth of that type in the sample exhibits evidence of mineralisation, or even whether a mean value is used to indicate the time of initial mineralisation. This therefore may influence the exact reference point that is being compared. Sunderland et al. (1987) attempted to overcome this problem by presenting their ages as a range over which initial mineralisation was observed in dentine. This included the youngest age at which mineralisation was first observed and the ages at which 50% and

100% of their specimens exhibited initial mineralisation. Smith (1991:156) stated that this is the '*best form in which such data can be presented*' and Hillson (1996:130) also added that cumulative distribution functions[5] '*should be the basis for the standards used in age estimation*'. However, from all of the studies reviewed only Sunderland et al. (1987) used a such a technique.

As the evidence of Kraus (1959b), Kraus and Jordan (1965) and Nomata (1964) all implied that deciduous teeth mineralise over a range of times, Lunt and Law (1974:606) suggested that any future research should include ranges and mathematical means rather than the single points in time that had previously been presented in the literature, as these '*will better reflect the variations in development*'. Kraus (1959b:1131) proposed that '*a mere statement of a mean or average time of initial calcification without indication of a range of variation in terms of standard deviation is useless and extremely misleading*'. Indeed, one single value completely ignores biological variation, which as discussed above can be quite pronounced. In addition, as initial mineralisation is often reported as occurring at a single point in time, for example 'week 15', one could argue that initial mineralisation could actually have occurred during the previous week and only been recorded the following week. This is not to say the data presented are wrong, but to question how the data are interpreted. This is also a disadvantage of recording the time of initial mineralisation in months rather than in weeks or days.

It is clear from the large range of ages of initial crown formation presented in **Table 3.3**, that even allowing for discrepancies caused by the fetal material and methods of aging and preservation, that dental development proceeds at different rates in different individuals, all of who may be considered 'normal' in every respect. It is therefore not possible to set a standard of initial crown formation for each week or month of life with the dictum that those not measuring up to the standard are 'abnormal'. However, as demonstrated above

[5] Cumulative distribution functions – the percentage of children attaining a particular developmental stage by a given age plotted as cumulative frequency graphs, from which mean ages of attainment are derived.

it is possible to develop chronological tables based on averages and ranges, which illustrate the normal biological variation of the human fetus.

Another point regarding what is actually being reported in the dental chronologies that have been reviewed above, is that while some researchers did not specify exactly what they were reporting (Broomell and Fischelis 1913; Churchill 1932; Churchill 1935), others recorded initial mineralisation as occurring at the first appearance of either dentine or enamel and others have recorded it as occurring at the first appearance of both. In addition while some researchers have reported that the difference between the onset of dentine and enamel mineralisation is negligible there are others who say that a difference definitely does exist.

Robin and Magitot (1861:643) recorded the appearance of '*the first cap of dentine which appears in each follicle*'. However, they go on to say later in their work that '*enamel begins to show itself at the summit of the dentine cap at the period when the cap measures about one millimetre in total height*', this would therefore increase the times of initial enamel mineralisation considerably (Robin and Magitot 1862:4). Karnosh (1926:29) stated that '*the growth of dentine is considerably in advance of that of enamel, and, therefore, a table of dentinification is not applicable to the study of layers of enamel*'.

Some researchers presented their observations of both enamel and dentine merged together as one value, for example Peirce (1877:400) presented the '*appearance of the cap of dentine and enamel*'. Kronfeld (1937:110), however stated that dentine formation always begins a '*short time*' before enamel formation and in the production of his table, mineralisation was considered to have commenced when '*the small cap of dentin and enamel first appeared on the tooth germ*' (Kronfeld 1937:123). Schour and Massler (1937) stated that previous experimental evidence in both animals and humans had indicated that the rate of enamel formation approximates that of dentine. They then stated that apposition begins with the formation of a tiny increment of dentin at the cusp tip

and that '*the formation of enamel begins a few days later*' (Schour and Massler 1940a:1921). Nomata (1964:74) also demonstrated that enamel formation occurs '*slightly after*' dentine formation in all tooth germs, unfortunately no exact times were provided. Nomata (1964:67) further suggested that the '*chronological difference between enamel and dentine formations is greater in the lateral incisors than in the central*'.

Again this non consistency in reporting initial mineralisation times could result in discrepancies in the comparison of dental chronologies. For example, Sunderland et al. (1987:168) recorded the '*histological evidence of mineralised dentine*' they then compared their data directly with other researchers who had recorded initial mineralisation in enamel, for example Kraus and Jordan (1965) who reported enamel measurements and Peirce (1877) who reported both dentine and enamel values together. However, no mention of this difference is made by Sunderland et al. (1987) even though they are directly comparing two different tissues.

3.6.2.d Human Error

As McCall and Wald in 1940 pointed out, until the work of Logan, Schour and colleagues, prenatal dental development had received relatively little attention from researchers. '*The basic work of early investigators established, to the satisfaction of all for nearly two generations, the modus operandi and chronology*' of prenatal dental development (1940:96). McCall and Wald also added '*that it seems to have been taken for granted by many that no important additional information was to be gained regarding prenatal tooth development*'. This does indeed appear to have been the case until the works Logan and Schour. However the void in prenatal dental developmental research then occurred again following these researchers and continued until the work of Kraus in 1959 and Kraus and Jordan in 1965 and in 1974 Lunt and Law finally suggested that the table produced by Kronfeld and Schour in 1939 be updated. This long tradition of not questioning the 'current' dental chronologies has meant that mistakes have also been perpetuated throughout the dental

chronology. Apart from possible errors in technique such as inaccuracies in specimen manipulation and orientation when measuring the CRL, reading the measuring equipment and possible clerical errors in recording the observations, there are also errors in the presentation of the chronologies themselves. Legros and Magitot (1880:3) admitted that in their previous work published in the *Dental Cosmos* the '*portion devoted to the origin and formation of the dental follicle was in many respects incomplete, and in some particulars erroneous*'*.* Throughout the literature, the reviewed authors have made errors in their work, sometimes even in citing their own work, (Hess et al. 1932; Peirce 1884; Robin and Magitot 1861; Schour and Massler 1940a; Wolfe 1935). These mistakes have contributed to the confusion of the initial mineralisation chronology and it appears that these mistakes have been perpetuated through these chronologies and have been accepted by their readers without question. For example, although the work of Sunderland et al. (1987) attempted to overcome the problems of using a pathological sample and also attempted to increase the accuracy of the ageing of their fetal specimens, the partial and incorrect conversion of data in their work has again contributed to confusion in the dental literature. Which unless the reader returns to the original source material runs the same risk of being copied and recopied in future work as previously demonstrated by the dental literature.

To conclude, from this review it can be seen that many general statements have been made regarding mineralisation of the deciduous dentition and that these have often been presented without adequate evidence or critical evaluation. In some cases statements have been presented as facts without reference to the original sources. In this way errors have been perpetuated unchallenged, through many editions and in texts by many authors.

From **Table 3.3** and the above discussions in **Sections 3.5**, it can be seen that the previous knowledge of deciduous crown formation varied considerably. The aim of this research is to attempt to improve this situation and to decrease this extensive range of deciduous crown initiation and completion times. This work attempts to update the previous knowledge by using specific regression formulae for each tooth type, derived from the observation of daily incremental

cross-striations in enamel. Furthermore instead of presenting a single initiation or completion time as done by previous authors, this research presents a mean average as well as a minimum and maximum range within which initiation or completion is most likely to occur.

CHAPTER 4: Incremental Nature of Enamel and the Neonatal Line

4.1 Introduction

As the underlying rationale behind this thesis utilises the incremental nature and structure of the deciduous enamel crown, the aim of this chapter is to present a description of the main incremental line that is examined in the histological section of this work, this being the neonatal line. This chapter commences with an introduction to the incremental nature of enamel which is followed by an account of the discovery of the neonatal line and how it was shown to be of neonatal origin; the structure and the position of the line is then described. Hypoplastic and hypomineralisation defects are then discussed in order to demonstrate the sensitive nature of the developing enamel. This chapter ends with a discussion regarding the differences between pre- and postnatal enamel.

4.2 Incremental Nature of Enamel

'*Completed enamel is like a tombstone, for on it is inscribed the history of the vicissitudes of the ameloblasts'*

(Schour and Kronfeld 1938:488)

Enamel is the product of appositional secretion by the ameloblasts, that is to say, the ameloblasts secrete enamel in layers one on top of another. This type of growth results in the formation of concentric layers that are delineated by 'growth' or 'incremental lines', as a result, these layers are therefore characterised by the regular and rhythmic manner in which enamel formation occurs. One form of incremental line results from the daily physiological rhythm in cellular activity; these are commonly referred to as enamel cross-striations. A longer period rhythm, spaced several days apart underlies the striae of Retzius

in permanent enamel; although these striae are much less prominent in deciduous enamel.

During formation and mineralisation, enamel and dentine are extremely sensitive to variations in metabolic processes, so much so that alterations in the internal environment of the body are often recorded as accentuated striae in the incremental layer that was developing at the time. The neonatal line in enamel has been reported as being an accentuated stria of Retzius (Andresen line in dentine), produced as the result of a disturbance of enamel formation and mineralisation and which occurs at the time of birth and during the immediate neonatal period. Only external factors such as caries, attrition or abrasion can erase these records from the teeth.

Incremental lines normally result from a daily physiological rhythm in cellular activity; certain additional systemic factors can also influence the cellular activity of the ameloblasts and this may result in an accentuated incremental line or layer which is easily distinguishable from the normal lines.

In general, accentuations of an incremental layer in enamel may be produced by two types of systemic effects:

1) constitutional or normally recurrent effects that occur during the normal functioning of the body (for example neonatal lines).

2) pathological effects, these in turn may be subdivided into defects of the internal and external environment.

Okada and colleagues described their research in English in a special issue of the Shanghai Evening Post in an article published during the War in 1943. Okada administered labels of various kinds (including lead acetate via intravenous injection) to rabbits and then observed the resultant incremental

lines. He demonstrated that within thirty minutes of an injection it was possible to identify a corresponding black line of lead between the dentine and predentine. Okada suggested due to the fact that the lead accumulated principally in the area that calcium accumulates in the hard tissue, that a chemical reaction had occurred between the phosphate, calcium and lead ions. Upon further investigation it was found that at the time calcium phosphate accumulated, the lead had replaced the calcium and precipitated as lead phosphate, which is more insoluble than calcium phosphate. Using the resultant lines from the intravenous lead acetate injections, Okada (1943:19) identified the occurrence of daily cross-striations in the dentine of rabbits and stated that '*white strata*' formed during the day and heavily stained strata formed during at the night.

Using sodium fluoride as a vital stain, Okada examined the growing permanent teeth of puppies, young pigs and young monkeys and again determined that there was daily incremental growth in both enamel and dentine. In one experiment, a Formosan macaque (*Macacus cyclopsis*) was injected intravenously with sodium fluoride three times over an interval of seven days. The enamel of its sectioned tooth revealed seven cross-striations between the lines produced by the sodium fluoride, indicating that they had formed at a rate of one per day. This is probably the first experimental evidence that enamel cross-striations represent daily increments.

As a result of the discovery of this daily rate, Okada (1943:19) suggested that the cause of these daily cross-striations was a '*vital phenomenon based on periodic changes in vivo*' and was not the result of a physico-chemical process as had been previously suggested. Okada then proceeded to identify the cause of this diurnal rhythm in the dentine of rabbits, by deliberately disturbing the physiological conditions that have such a daily rhythm, for example, light, nutrition and sleep. He found that extended periods of drug induced sleep resulted in '*unusually deep-stained zones*' corresponding to the period of sleep (1943:20).

Okada suggested that changes in the acid base equilibrium of the body fluids may be responsible for these cross-striations, so he measured the alkaline

reserve[6] in the blood plasma and found that the alkaline reserve decreased during the day and increased at night '*manifesting an unmistakable periodic variation between day and night*' (1943:21). This suggestion was further supported by artificially created changes in the acid base balance which resulted in either hypo- or hypermineralised lines in dentine, with an increased alkaline reserve manifesting as a deep stained line (night) and a decreased alkaline reserve manifesting as a white stratification (day); so acidosis was found to inhibit mineralisation and vice versa. Okada also investigated the relationship between calcium plasma levels and incremental lines, again using rabbit dentine. His results indicated that distinct changes occurred in the calcium content in blood plasma and that these changes corresponded to lines in the dentine. In the daytime Okada stated, the calcium content was constant while at night it showed a marked decrease.

Okada also measured the carbon dioxide capacity of the blood plasma in rabbits during pregnancy and found that as in the case of humans it decreases towards the end of pregnancy to a minimum on the day of birth and then after birth it is rapidly restored. In modern terms, PCO2 levels are now measured in kilopascals (Kpa) of pressure. Normal levels are around 4.5–5.8 Kpa but as PCO2 levels rise (often because of kidney failure or lung disease) blood pH falls and becomes more acidosed. This decreasing carbon dioxide capacity (increasing acidosis) as Okada (1943:23) described it, is reflected in the dentine as the lines become fainter and fainter until the day of birth when a '*sharp white stratification*' indicating decreased mineralisation can be observed in the dentine, which is then followed on the first night after birth by a deep stained striation (increased mineralisation). This discovery of the formation of a parturition line in rabbit incisors formed while the mother was in labour and giving birth was attributed to metabolic acidosis due to the physical effort of labour, but interestingly, humans infants are often born blue and cyanosed and the acidosis associated with this, together with the low calcium levels at birth, may well contribute to the appearance of the neonatal line.

[6] Alkaline reserve – amount of buffer compounds in the blood capable of neutralising acids.

In 1931a, Swanson described several regularly reoccurring and prominent accentuated striae of Retzius, which he stated might be caused by the decreasing amounts of sunshine during the winter months. One particular accentuated stria which occurred at about one year of age he suggested was possibly caused by the '*nutritional disturbances associated with weaning*' (1931a:826). However, the process of weaning varies between individuals, whereas the 'one–year' line described by Swanson showed a high degree of chronological constancy. Weaning is also a gradual process rather than a sudden event and the appearance of a prominent line at one year is unlikely to be indicative of such a gradual process. In 1941 Massler et al. using 'tooth ring analysis' attempted to define a number of 'growth rings' which each marked a specific stage in normal crown development and which corresponded to a specific period in childhood development. For the deciduous enamel crown these were the prenatal period which was demarcated by the neonatal line and the infancy period which was demarcated by the 'infancy ring' (see **Section 3.3.4.c**), however like the 'weaning line' suggested by Swanson in 1931a, with the exception of the neonatal line which has been firmly established as occurring during the neonatal period, these additional 'rings' and lines have not survived the test of time.

4.3 Neonatal Line

'*Birth is the most profound change in environment and nutrition which man experiences from conception to death*'

(Kronfeld and Schour 1939:20; Schour and Kronfeld 1938:471)

The first reference to the neonatal line in the literature was by Ruston in 1933. Rushton (1933:170) was investigating the '*fine contour lines of the enamel of milk teeth*' and described the existence of several such lines in his histology sections. He commented that there are usually at least one or more of these lines present in the enamel, the most obvious of which are '*dark brown by transmitted light*' and which appear under a low magnification '*to be sharply*

defined and of practically no thickness' (1933:170). Using a higher magnification Rushton stated that the enamel prisms do not appear to be broken and they do not change direction or appearance as they cross these lines, the only visible change appeared to be that the prism outline was sometimes '*a little irregular*' (1933:170). He added that these lines are visible because of the '*increased darkness of the intervals between the prisms, producing an aspect like a rope*' (1933:170) which is about 10-20µm thick.

Rushton mentioned that similar lines had previously been described by von Ebner in 1905 and Rygge in 1916. Evidently von Ebner (1905) had stated that these lines consisted of incompletely formed enamel due to an arrest of enamel growth at an early stage of development and which was probably due to a physiological cause. However, Rushton pointed out that an arrest in enamel growth would not leave any trace unless some alteration in the quality of the enamel had also occurred. Rygge (1916) on the other hand had stated that these lines were more highly mineralised than the surrounding enamel and that their function was to strengthen the enamel structure. Rygge stated that while enamel could be stained with alcoholic fuchsin, these lines even in very young teeth could not be stained, he also added that the lines were completely dissolved by acids. Rushton suggested that as the colour of these lines changed from dark to light when the microscopic light source was changed (substitution of dark ground illumination for transmitted light) that the colouration of the line was not due to a pigmentation but rather to a sudden difference in the refractive indices of the surrounding enamel. Rushton went on to say that as the greatest contrast in the refractive indices lay between the prisms and the interprismatic substance at the line, that this indicated that the interprismatic substance in this area was either more or less highly mineralised with respect to the prisms in this area than elsewhere in the enamel.

After investigating '*a number*' (1933:170) of ground sections of deciduous teeth, Rushton noted that there was a regularity in the distribution of some of these lines, although not all of them. As the first of these lines was in a similar position in the enamel in each tooth type Rushton suggested that it occurred at the

same point in the life history of each tooth type. He noted that the position of the first line in different tooth types was proportionally nearer to the dentine in those teeth which were formed later and it was nearer to the enamel surface in those teeth which were formed earlier. He observed teeth from several individuals and concluded that this line appeared to have been formed at the same point in the life history of all of the individuals. As the amount of enamel formed at the time of the appearance of the line corresponded well with the amount of enamel known to be formed at the time of birth, he concluded that this line represented the effect of birth or the '*uncertain period immediately*' following it and he identified this line as the '*birth line*' (1933:171).

In contrast to the 'birth line', Rushton observed that the subsequent lines which were sometimes present, seemed to occur much more randomly and in addition they were not located in the same positions in homologous teeth from different individuals in the same way that the 'birth line' was. However, in similar teeth from the same person they were found in similar positions and even in different teeth from the same individual they occupied positions which corresponded chronologically with each other. Rushton suggested that this indicated that these lines may occur as the result of some '*noteworthy constitutional disturbance*' (1933:171). He compared these enamel lines with the Harris lines, which had been observed in long bones. In 1923 Harris had reported his observations of transverse lines in the metaphysis of long bones to 'The Anatomical Society of Great Britain' and he had attributed these lines to the arrest of growth but with continued mineralisation, which was then followed by the resumption of growth and normal mineralisation. Harris had shown these lines to occur in similar circumstances as the lines in the enamel that Rushton had observed (Harris 1933). In order to test this theory Rushton examined several sections through first and second molars from individuals with known medical histories. Unfortunately he was unsuccessful in finding any correlation between the two. He stated that '*it proved impossible, however, regularly to trace any chronological correspondence between these lines and the events mentioned in the case histories*' (1933:171). However, Rushton did suggest that if the rate of enamel formation was more or less consistent, '*then one must*

conclude that, whatever kind of disturbance gives rise to these random lines, it is not necessarily memorable or much of an outward sign' (1933:171).

Although Rushton included photographs of these ground sections alongside a very brief medical history, he did not provide any further information about how he had calculated the individual crown formation times or the estimated age of the individual at the time of the illness. Furthermore, since a scale was not provided, it is even more unfortunate as the formulae developed later in this work cannot be applied to these particular case histories. However, it is clear from Rushton's photographs that both neonatal and 'stress lines' are present and that the medical history provided was insufficient to really allow any correlation between the ground sections and the history (see **Chapter 7**).

In 1936, Schour examined 250 demineralised serial sections and 100 ground sections of deciduous teeth from several sources, (including Kronfeld's pathological sample, see **Section 3.4**), in addition he examined serial sections from two full-term stillborn infants. Schour described a '*distinctive incremental line*' (1936a:1946) in the enamel and a similar corresponding line in the dentine that was present in 90% of the teeth that he examined. However, in the serial sections the teeth were demineralised and the enamel lost and therefore the neonatal line in the enamel was also lost. This means that only the 100 ground sections could have been examined for the presence of the neonatal line in the enamel. Schour added that this line is present in both the enamel and dentine when a ground section is prepared that passes through the highest points of the EDJ, the point at which enamel formation and mineralisation begins. Massler et al. (1941:45) later reported that the neonatal line can be observed in '*virtually all deciduous teeth if sections are carefully prepared in a sagittal plane*'.

The location of this line in the enamel appeared to be at a consistent position from the EDJ in each tooth type and Schour (1936a:1947) suggested that the apparent consistency and characteristic position of this line indicated that it was caused by some '*constant and universal condition*'. As the line was present in

the teeth of children only a few months old, Schour suggested that the cause of the line occurred at birth or very soon after it. Furthermore the position of this line also corresponded to the amount of enamel that had developed in different tooth types at the time of birth. So although Schour did not refer at all to Rushton's original 1933 work, he reached the same conclusions as Rushton had done and he suggested that this '*distinctive incremental line*' (1936a:1946) was formed at birth or very soon afterwards. Due to its close association with birth, Schour renamed Rushton's 'birth line' the '*neonatal line'* (1936a:1950).

In order to provide further quantitative proof of the neonatal origin of this '*distinctive incremental line*' (Schour 1936a:1946), an experimental study of the rate of enamel formation by means of injections of sodium fluoride into a living human juvenile was conducted (Schour and Poncher 1937). Each injection produced a '*sharply accentuated incremental line*' (Schour 1936a:1951) in the enamel forming at the time of the injection. Unfortunately, no explanation is provided regarding the actual mechanism behind this resultant 'injection line'. From average measurements between the '*distinctive incremental line*' (1936a:1946) and the 'injection line' the approximate amount of daily enamel apposition was determined to be 4µm per twenty-four hours. Using this information it was then possible to firmly establish that the '*distinctive incremental line*' (1936a:1946) previously described by Schour actually did occur on the infants birthday and that it was in fact of neonatal origin.

Schour (1936a:1953) stated that '*it is not surprising that the severe metabolic disturbances which the new-born infant experiences during the time when it ceases its intra-uterine existence and has to adjust itself to the changes incident with extra-uterine life should leave their mark in the teeth*'. This is supported by the fact that under normal conditions, the newborn infant usually regains its birth weight by the end of the second week of extra-uterine life (Wright and Parkinson 2004). This loss in weight during the neonatal period and the resultant temporary sub-nutrition are well known manifestations of birth. In addition to Schour's identification of the neonatal line in deciduous teeth, Harris (1933:25) identified neonatal changes in the long bones, which appeared as

'*lines of arrested growth due to the relative starvation in the first week of life*'; and Sontag (1938:1256) described the presence of neonatal striae in infant tarsal bones, which he suggested are caused by the '*slowing or cessation of growth at birth, perhaps resulting from the shock of birth itself or from chemical readjustments that occur in the first few days of postnatal life*'.

The identification of the neonatal line and its consistent and characteristic position accompanied by quantitative proof of its neonatal origin allowed the development of further investigations into deciduous crown formation times and the sequence of deciduous dental development. From this 1936 work, Schour (1936a:1954) had successfully established the neonatal line as a '*permanent biologic landmark which can be used for the determination of the amount and quality of the enamel and dentin laid down before and after birth*'.

After successfully proving that the neonatal line was of neonatal origin Schour proceeded to investigate this line in more detail. In 1938, Schour working with Kronfeld examined demineralised and ground sections from the deciduous teeth of a female infant who although born after a normal delivery at term, failed to develop properly or to show a normal response. On admission to hospital with an infection of the upper respiratory tract, she was diagnosed with an injury of the brain that had evidently been sustained at birth. The infant later developed bronchopneumonia to which she succumbed at the age of seven months and five days (218 days).

Radiographs were taken and then each jaw was divided in the midline, one half was demineralised, sectioned and stained; the other half was made into ground sections. This way it was possible to compare the corresponding teeth from the same individual using both demineralised and the ground sections. For the study of the enamel neonatal line the ground sections were examined since most of the enamel had been dissolved during the preparation of the demineralised sections.

The appearance of the enamel neonatal line in this individual was reported as being more severe than the corresponding line in the dentine; in fact the neonatal injury had been so severe in the enamel that excepting the lower canines and second molars, amelogenesis had been permanently arrested at birth. This arrest in ameloblast activity resulted in the neonatal line coinciding with the enamel surface. Only in the lower canines and second molars had the ameloblasts survived and managed to continue functioning past the neonatal line. Cervical to the neonatal line, the cells of inner enamel epithelium of the enamel organ, which at the time of birth were still differentiating, had been unaffected by the injury and this resulted in the formation of a circular ridge of postnatal enamel adjacent to the hypoplastic area.

Throughout the deciduous dentition of this individual the neonatal line was extremely prominent both in the enamel and dentine. The neonatal line was reported as being a '*pathologically accentuated*' (1938:489) '*dark line in the enamel dividing the prenatal and postnatal portion*' (1938:475) and under high magnification the enamel prisms in this area were seen not only to change colour but also to deviate markedly from their course. Schour and Kronfeld (1938:473) stated that '*it is obvious that the greater the disturbance at birth, the more accentuated*' the neonatal line will be. In addition the prenatal enamel was reported as being '*uniform and typical in structure and thickness*' (1939:22), while the postnatal enamel showed a '*dark discoloration*' (1939:22).

The position of the neonatal line in the dentine of this individual was also unusual and was reported as being '*slightly above the level of the normal full term position*' (1938:477) which Schour and Kronfeld suggested was indicative of the infant being born slightly prematurely, they added that this suggestion was further corroborated by the slightly subnormal birth weight. They suggested that the neonatal line would be more prominent in the teeth of prematurely born children than in infants born at term because of the relatively greater nutritional difficulties and other disturbances usually encountered by premature infants. They added that '*the more difficult it is for the organism to make the adjustment from the sheltered intrauterine life to the independent postnatal existence, the*

more pronounced will be the zone of poorly formed and calcified enamel and dentin apposed during this period' (Schour and Kronfeld 1938:473).

Schour and Kronfeld (1938:489) stated that the neonatal line was '*a manifestation of the physiologic neonatal arrest of growth*'. Rushton (1939:7) also stated that during the neonatal period '*the growth of enamel is believed to be retarded or arrested'* and in 1941 Massler et al. (1941:45) suggested that the neonatal line '*probably represents a line of arrested growth rather than a line of disturbed calcification*'. This concept of a neonatal arrest in growth was also raised more recently by Mishra et al. (2009:S105).

In 1937, Schour and Massler had very briefly alluded to a period of 'neonatal arrest' and in fact this was so brief that it only consisted of one sentence: '*Analysis of jaws of ten children, one hour to 6 months old, showed neonatal arrest in growth averages 14 days*' (Schour and Massler 1937:350). In their 1939 work Kronfeld and Schour (1939:23) reported that normally the neonatal arrest period was '*from ten to fourteen days duration*', however no further details regarding how this number was established are presented and the reader is referred back to the one sentence from the 1937 Schour and Massler abstract. In the current 1938 work Schour and Kronfeld (1938:482) stated that in this individual the amount of postnatal dentine was '*less than normally would be expected*' for a seven month old infant and they suggested that the dentine development '*seemed to be about two months late*' (1938:482). Although born at term and the results of their radiographic observations had previously illustrated that the state of mineralisation corresponded to that of a child of approximately six months old, it appears from their 1939 work, that Kronfeld and Schour came to this conclusion because the thickness of the prenatal and postnatal enamel was about the same and this indicated that a temporary arrest or delay in postnatal development had occurred. After refining the period of neonatal arrest using 'tooth ring analysis' (see **Section 3.3.4.c**) they reported an average arrest of 71.2 days. Which when compared to the usual length of neonatal arrest '*which normally lasts about two weeks*' (1938:482) is considerably longer, however Schour and Kronfeld suggested that this arrest

period has been '*prolonged to two months because of the injury at birth*' (1938:482). They suggested that the neonatal injury of the brain in this case may have produced in a prolonged state of malnutrition which in turn affected the ameloblasts; they added that infants suffering from the same type of brain injuries are often extremely undernourished.

Although no further details are provided in this 1938 paper regarding how or why they arrived at this conclusion, from the table that they included in this work it appears that they had taken measurements from the tip of the dentine neonatal line to the tip of the pulp horn, then divided this measurement by the rate of appostion that was presented in the 1937 work with Massler (this work presented the rate of apposition for the central incisor as ranging from 5-8µm). For example for the central incisor, the distance from the tip of the dentine neonatal line to the tip of the pulp horn was 1080µm divided by the daily rate of appostion (8µm) gives 135 days of active postnatal dentine formation. If this is then subtracted from the number of days of life (218) the result is a neonatal arrest time of 83 days (not the reported 82 days). The average for all five tooth types was reported as being a neonatal arrest of 71.2 days. However if the same distance from the tip of the dentine neonatal line to the tip of the pulpal horn (1080µm) is divided by the age of the individual (218 days) then appositional rate for central incisors should be 4.95µm rather than 8µm, which is more similar to the average 4µm daily rate of apposition reported by Schour (1936a) and Schour and Poncher (1937) and even Schour and Kronfeld earlier in the same paper (1938:473) as opposed to the 8µm daily rate that they used. However in this case, this 4µm rate clearly does not work as this results in a postnatal dentine formation period of 270 days!

Although the occurrence and formation of the neonatal line has been attributed to the abrupt change in environment and nutrition that the new born experiences at birth, attempts to determine the actual structure of the neonatal line have resulted in several conflicting conclusions.

Rushton, reported that the contour lines he had been investigating were '*dark brown by transmitted light*' (1933:170) and '*light by reflected light*' (1939:5) and that they appeared under a low magnification '*to be sharply defined and of practically no thickness*' (1933:170). Using a higher magnification he stated that the enamel prisms do not appear to be broken and that they do not change direction or appearance as they cross these lines, the only visible change seemed to be that the prism outline is sometimes '*a little irregular*' (1933:170), he added that these lines are visible because of the '*increased darkness of the intervals between the prisms, producing an aspect like a rope*' (1933:170) which is about 10-20µm thick. In 1939 Rushton used birefringence[7] analysis to examine the neonatal line in the enamel of 50 ground sections in order to review his original idea of a change in refractive index within the line. He had observed that the neonatal line demonstrated a high negative birefringence[8] of the prisms and little or no birefringence of the interprismatic substance. From this he concluded that the enamel prisms became more highly mineralised as they crossed the neonatal line and that '*during the neonatal arrest of growth calcification of prisms proceeds to a high degree but calcification of the interprismatic substance is low*' (1939:9). Rushton also reported that the enamel prisms did not change direction as they crossed the neonatal line.

Schour and Kronfeld (1938:472) stated that the neonatal line in enamel is a '*pronounced stria of Retzius*'. According to Noyes (1938:106), writing in the same year, '*a stria of Retzius represents a poorly calcified portion of the enamel, because it is made up of the relatively less calcified components of the enamel rod*'. Massler et al. (1941:45) reported that the enamel neonatal line appeared '*dark or hypocalcified*' under a higher magnification and Schour and Kronfeld (1938:475) observed that as the enamel prisms crossed the neonatal line they not only changed colour but they also deviated from their original course.

[7] Birefringence – or double refraction, is the decomposition of a ray of light into two rays when it passes through certain anisotropic (directionally dependant) materials such as for example a crystal of calcite.

[8] Negative birefringence – fully mineralised enamel exhibits negative birefringence. The '*more negative the birefringence with respect to the prism direction the more complete the calcification*' (Rushton 1939:2).

Sognnaes (1949:560), whilst studying demineralised paraffin sections of deciduous teeth, observed a '*heavy line*' which corresponded in '*appearance and position to the neonatal line*', this is one of the first accounts of the neonatal line being observed in the 'enamel' of a demineralised section. Sognnaes (1949:560) stated that like the fainter striae of Retzius '*it is evident that these incremental lines owe their appearance to the morphology of the main organic elements of the enamel (the prism sheaths)*'. It is the thickened portions or '*heavy zones*' of the prism sheaths as they cross the neonatal line which gives the prisms their '*bead-like*' appearance (1949:563). This description is similar to the one previously presented by Rushton (1933:170) who described the prism outline as being sometimes '*a little irregular*' and that this resulted in the '*increased darkness of the intervals between the prisms, producing an aspect like a rope*'. Gustafson and Gustafson (1967:96) also reported having observed a rare type of Retzius line that was produced by '*widening of the interprismatic substance and narrowing of the prisms*'. However, unlike Gustafson and Gustafson, Sognnaes stated that the prisms passed straight through the neonatal line and did not appear to show any evidence of bending.

Jansen and Visser (1950) whilst investigating the permeable structures in enamel using fluorescent dye, stated that the Retzius lines they had observed in ground sections from dogs were rich in organic material and were formed from thickened parts of prisms sheaths, they also stated that they could find no '*deflection in the prism bodies*' (1950:629).

Using microradiography, Crabb (1959:120) examined 130 ground sections from humans ranging in age from a fetus of 32 weeks to a child of seven years and he identified the neonatal line as being '*relatively radiolucent*' which he stated confirmed its hypomineralised nature. Later the same year Allan examined ground and demineralised sections produced from the same teeth from the same individuals. These sections had been made from 52 deciduous teeth removed at 17 post-mortem examinations, ranging in age from a fetus of 26 weeks to an infant of 78 postnatal weeks, (crown-rump measurements were used to establish age). Using both polarised light and microradiography Allan

came to the same conclusion as Crabb, that the neonatal line is hypomineralised. Silness (1969) also using microradiography and light microscopy examined ground sections from 25 deciduous teeth and confirmed the findings of Allan and Crabb regarding the radiolucent and therefore hypomineralised nature of the neonatal line.

Silness (1969:102) reported a '*widening or thickening*' at the site of the neonatal line and stated that this was due to '*thickenings of the prism boundaries*' (1969:104) this observation is in agreement with Sognnaes (1949) who had also reported a thickening of the prism sheaths at the line. Silness suggested that it was also possible that reduced mineralisation or lack of mineral in the peripheral parts of the prism may increase the width of the radiolucency of the prism markings which form the neonatal line. He also reported that at its cervical termination the neonatal line did not reach the enamel-dentine junction. Jackobsen (1974:101) disagreed with Silness on this last point and stated that the '*neonatal line would quite often reach the enamel dentine junction*'.

Allan (1959:1102) found that under polarised light the striae showed '*well-defined variations in the mineral content*' along their length. Negative lines were seen entering the isotropic and positive zones and positive lines penetrated negative zones. Although Allan suggested at first that the variations in the strength of birefringence along the neonatal line may be related to alterations in prism direction and crystallite orientation, upon further investigation he felt that although he had seen these changes, they were however '*insufficient to account for the appearances*' that he had observed (1959:1105). Microradiographs of the same ground sections also showed lines of low and high absorption and Allan (1959:1124) suggested that these sharp variations were due to the '*amount of mineral matter present along these lines*'. Stained demineralised sections from the same teeth also showed a variation of staining along the striae and Allan (1959:1124) concluded that the mineral variations were due at least in part '*to the quantity and quality of the organic matrix deposited during this period*'. These corresponding results also demonstrated that there was concurrence between the methods that Allan had used.

Gustafson and Gustafson (1967:97) considered the neonatal line to be a type of pathological Retzius line which was sometimes '*very insignificant*'. They stated that Retzius lines are usually hypomineralised, however they also reported having observed rare cases where the lines were hypermineralised for a certain distance while the rest of the line was hypomineralised, they suggested that on these occasions this variation in mineralisation of simultaneously formed prisms suggested that a '*local factor must play a role*' (1967:95). They also stated that striae of Retzius are caused by bending and changes in the direction of the enamel prisms and that sometimes '*disturbances in the formation of the prism segments can be seen*' as the prisms cross the line (1967:90).

The report by the two authors above, regarding the change in appearance along the length of the neonatal line may be due to the fact that the line was being observed mid way through its mineralisation. Furthermore it could be possible that all of these descriptions of the neonatal line are correct, just that the line has been observed at different stages during dental development and the observations were made at different places along the length of the line.

Weber and Eisenmann (1971), studying the neonatal line in 25 deciduous teeth using phase contrast microscopy, microradiography and transmission electron microscopy, stated that the neonatal line appeared to have the same 'zigzag' structure as the striae of Retzius, similar to that previously reported by Gwinnett (1966) and Poole (1967). From observations of these 25 longitudinally sectioned teeth, using transmitted light microscopy, they described the neonatal line as a '*dark linear band*' which was about 20-30µm in width (1971:376). They also mentioned that where sections had been cut in a more oblique plane, for example in the gnarled enamel, the '*line had a more diffuse appearance as well as apparently greater lateral dimensions*' (1971:376). They stated that although they did not observe any changes in prism orientation, continuity of the prisms did seem to be '*temporarily interrupted at the line*', however as Whittaker and Richards pointed out that this could be the result of the obliquity of the section. Using phase contrast microscopy the line was observed to have a 'staircase'

configuration, which appeared to be built up of a series of prominent dark cross-striations and segments of prism boundaries, both of which were 1µm in width. Using microradiography, the line as previously demonstrated by Crabb (1959) and Allan (1959) appeared as a radiolucent band with diffuse peripheral margins, again indicating its hypomineralisation. Using transmission electron microscopy Weber and Eisenmann (1971), remarked that the neonatal line was surprisingly inconspicuous, they found that it appeared to be missing from several of the sections that had previously exhibited a line using the other observation techniques. The line, when located, appeared as a thin crystal deficient region running obliquely across the enamel prisms. The line was continuous with the neighbouring prism sheaths and was similar to them except that it was slightly thicker than the typical prism sheath. The crystal deficient region was not always continuous across the prisms. Some prisms appeared to cross the line undergoing a dramatic change in orientation as they did so.

Weber and Eisenmann (1971) concluded that there was little correlation between their observations of thin ground sections, using phase contrast microscopy and microradiography and the descriptions of previous researchers using light microscopy. They found no evidence of thickening of the prisms boundaries or prism sheaths and no gross bending of the prisms occurred. There was however some constriction of the parts of the prisms within the line, although they also stated that the constriction of the prism was not compensated for by an expansion of the adjacent prism boundaries but rather by the adjacent prism. However, as mentioned earlier, their description of the 'zigzag' structure of the striae of Retzius, was similar to that previously reported by Gwinnett (1966) and Poole (1967). This lead Weber and Eisenmann (1971) to propose that the light microscope and ultrastructural characteristics of the neonatal line described in their work may also apply to other Retzius lines. However Gwinnett had reported that this configuration did not represent a 'typical' Retzius line and that it only occurred in 15% of the sections that he examined.

Weber and Eisenmann (1971:378) concluded that the '*ultrastructural basis for*

the neonatal line may be no more complex than a localised change in configuration of enamel prisms along with a possible reduction in the concentration of crystals', although they added the possibility of prisms terminating at the neonatal line could not be excluded. They finally stated that the previous light microscopic and microradiographic descriptions of the neonatal line, such as the thickening of prism boundaries and excessive lateral extension of the line are products of section thickness and optical artefacts.

Whittaker and Richards (1978) examined longitudinal and transverse sections from 52 deciduous and permanent first molars. They established that the neonatal line was present in all of the teeth that they examined in both the longitudinal and transverse sections; this 100% occurrence rate corresponded well with Schour's (1936a) figure of 90% which was obtained from his original investigation of 250 demineralised serial sections and 100 ground sections. Although most researchers had previously observed the neonatal line in longitudinal sections Whittaker and Richards also demonstrated that it was equally apparent in transverses sections.

Using scanning electron microscopy at a low magnification, Whittaker and Richards (1978:45-46) described the neonatal line as a '*discrete dark structure*' which seemed to mark an area of change in '*orientation of prisms occurring between the prenatal and postnatal enamel*'. At higher magnifications it was noted that structural variation was also visible in the area of the line. The majority of prisms appeared to be continuous as they crossed the neonatal line; however in some of the sections this continuity was dubious, although this was only noticeable in sections that were oblique to the line of prism direction. In longitudinal sections, the prisms were observed changing direction in a 'zigzag' manner as they crossed the neonatal line, with the prisms in the postnatal enamel remaining parallel to those of the prenatal enamel. Only in a few cases did the prisms show a major change in direction at the line and fail to regain their original orientation. At the point where the prisms crossed the line they were reported as often being constricted and the surrounding interprismatic areas were noted as being correspondingly widened. It therefore appears that

the neonatal line was not only produced by a directional change but also by a structural change within each prism at this point, consisting of a clearly defined interruption about 0.2µm wide running transversely across the prisms. Whittaker and Richards (1978:46) stated that this '*0.2µm wide sharply-defined cross band of the prisms results from the limited period of severe physiological disturbances occurring at birth*'. Although it is not precisely clear whether these defects of the line extend around the prisms or throughout their entire thickness, the slight displacement of some of the prisms through the line suggested a defect within the substance of the prism, however Whittaker and Richards added that this may also have been caused by sectioning or polishing.

On the postnatal side of this 0.2µm wide line Whittaker and Richards identified a wider diffuse zone of about 15-16µm, where the enamel crystal density was reduced. The total length of affected prism at the neonatal line is therefore of the order of 16µm. Whittaker and Richards suggested that the previous reports of the thickness of the neonatal line may have been exaggerated due to the thickness of the section or by optical artefacts. Whittaker and Richards (1978:46) suggested that this 16µm wide diffuse zone may represent a period of growth of some three to four days whereas '*reports using light microscopy have suggested a 14-day period after birth for production of a neonatal line*'. It is unclear where this 14-day period of formation could have originated from, no other mention of this can be found in the literature. The only 14-day period mentioned with regards to the neonatal line is the 'neonatal arrest period' of Schour and Massler (1937) and Kronfeld and Schour (1939), however in these studies the authors stated that the enamel formation was arrested (see above for further details).

Gustafson and Gustafson (1967), had also described the neonatal line as being sharply defined on its dentine facing side but less sharply delineated towards the enamel surface and Silness (1969:94-95) had observed specimens in which the neonatal line was '*fairly well delimited towards the prenatal enamel*', while the '*delimitation towards the surface was, however, poorly defined*'. Whittaker and Richards (1978:47) stated that '*it seems clear that some disturbance in*

physiological activity of the ameloblasts occurs at the time of birth and continues to a diminished degree in the succeeding few days'. This sharply defined cross-band of prisms on the prenatal side of the line they suggested would be detectable by scanning electron microscopy in developing deciduous enamel before the subsequent more diffuse neonatal part of the line is completed. This may be of value for forensic purposes, for indicating whether an infant lived, even for a short time after birth. With light microscopy, this is currently only possible if the child lived for several weeks after birth, (Whittaker and Richards, 1978).

Although a variation in the appearance of the neonatal line did appear to exist between individual teeth, Whittaker and Richards were unable to identify a pattern behind this variation in the 52 teeth that they observed. Previous workers had attributed the appearance of the neonatal line to bending of the prisms, however although Whittaker and Richards findings supported this they are also in agreement with those authors who stated that the neonatal line was caused by a change in prism shape. It is not clear, however why in some teeth prisms were observed to deviate from their course as they crossed the line and not resume their original path, while in other teeth the prisms followed a 'zigzag' pattern across the line and then resumed their original course.

Previous researchers have described changes in width of the prisms as they cross the line and have attributed this to the expansion of adjacent prisms or to increased spacing between them. Whittaker and Richards confirmed that some prisms are constricted but that this is not a constant feature. They suggested that this appearance is likely to be due to the normal undulations previously described by Osborn (1967), which would produce this appearance as the prisms bend through the region of the line.

Observations by Whittaker and Richards (1978) are also consistent with reports of increased porosity both in neonatal and Retzius lines, as demonstrated by the affinity for fluorescent dyes by Jansen and Visser (1950) and by the

microradiographic and staining studies of Allan (1959). However, Whittaker and Richards were unable to definitely confirm the view that neonatal lines resemble striae of Retzius, as no comparative study has yet been undertaken observing the striae of Retzius using a method similar to the one used by Whittaker and Richards (Kodaka et al. 1996). Newman and Poole (1974) have suggested that differences may exist between striae in the same tooth, however until a neonatal line and Retzius line are examined and directly compared using scanning electron microscopy the answer will remain uncertain.

More recently Sabel et al. (2008), used polarised light microscopy, microradiography, scanning electron microscopy and x-ray analysis to investigate the neonatal line in 30 exfoliated deciduous incisors, 5 molars and the tooth buds from 19 deceased infants. In polarized light the neonatal line was observed as a '*distinct, positively birefringent band*' (2008:957) that appeared to have a more porous structure than the rest of the enamel. In microradiographs the '*thin radiolucent band*' (2008:957) was again interpreted as the neonatal line being hypomineralised with regards to the surrounding enamel. Both of these observations confirmed the work of previous researchers.

Using scanning electron microscopy the enamel prisms were seen to reduce in diameter as they crossed the line with the more narrow diameters continuing through the postnatal enamel; with the mean prenatal prism being 5.35µm and the postnatal prism being thinner at 4.80µm, a difference of 0.55µm. Sabel et al. (2008:962) suggested that this may '*reflect a not yet fully mineralised prism*' or it may '*reflect a consisting modification of the ameloblast at the neonatal line, resulting in a smaller diameter of the prisms*'.

In some specimens Sabel et al. (2008:958) observed that some of the prisms changed direction as they crossed the line and at a higher magnification this was reported as a '*disturbance in the enamel structure organization*'. In a few of their tooth bud specimens they also observed the 'stair case' configuration of the line that had previously been reported by other researchers. A change of the direction of the prisms was also observed at the neonatal line, with the prisms

changing orientation by about 6 degrees, although, in some sections a divergence of up to 20 degrees was observed.

X-ray analysis was used to investigate the inorganic constituents of the neonatal line, it showed that there were no '*marked or consistent variations around the neonatal line*' (Sabel et al. 2008:960). There was however a slight decrease in the weight percent of calcium in the line of some of the exfoliated teeth, however the calcium:phosphorus ratio remained constant. There was also a gradual decrease of calcium and phosphorus towards the enamel surface.

After their extensive investigations, Sabel et al. (2008:962) concluded that the neonatal line is an '*optical phenomenon due to alterations in height and degree of mineralisation of the enamel prisms*'.

Mishra et al. (2009) have recently made the interesting observation that the neonatal line acts as a barrier to the progression of carious lesions. Using scanning microradiography, these authors concluded that rates of demineralisation in pre- and postnatal enamel were generally about the same; however the rate of demineralisation was much lower in the close vicinity of the neonatal line. Mishra et al. suggested that this reduction in the rate of mineral loss in the vicinity of the line was caused by a slowing down of enamel matrix formation which resulted in a change in the orientation of the enamel crystallites. In addition, the enamel which formed at this time may have continued to mature during the neonatal arrest period and therefore reach a higher degree of mineralisation than that formed before or after it. This slowing of the rate of formation would also reduce the carbonate:magnesium ratio to the calcium:phosphate component of the hydroxyapatite. The combined result, the authors argued, resulted in a less acid-soluble region, which paradoxically, may be able to mature to a greater degree of mineralisation than the enamel either side of the neonatal line (Mishra et al. 2009). However, this suggestion is not in agreement with the previous reports of the neonatal line being hypomineralised. Mishra et al. (2009) concluded that the decreased rate of enamel matrix

formation around the time of birth may explain why the neonatal line appears to act as a barrier to the progression of caries.

It could be that all of these conflicting appearances of the neonatal line are actually correct, but that the line was being observed at a different stage of its development, at different locations or even in different tooth types.

So in conclusion, the literature regarding the structure of the neonatal line has often resulted in conflicting views, however, the consensus seems to be that the neonatal line is formed as a result of a localised decrease in mineral content, which is accompanied by a decrease in prism diameter and a bending of the prisms as they cross the line.

4.3.1 Incidence, Location and Width of the Neonatal Line

The neonatal line is a normal incremental feature of enamel, corresponding to a stria of Retzius produced at birth, which extends more or less obliquely from the enamel-dentine junction cervically, to the enamel surface occlusally. The neonatal line has been described as being the border between the prenatal and postnatal enamel. Although research has been carried out regarding the structure and cause of the neonatal line, the actual location of the line is not so well documented. Whittaker and Richards (1978:45), observed the neonatal line in transverse sections and as would be expected they reported that the distance of the line from the enamel-dentine junction was '*dependent on the level of the section but, nearer the cervical margin, the concentric line was close to the junction and crossed the region of the enamel tufts*'. This is not really surprising as Schour whilst working with Poncher (1937:722) had previously referred to the neonatal line as a 'neonatal ring' and so the nearer to the cervical margin the section is made the closer the line will be to the enamel-dentine junction.

The enamel neonatal line is found in deciduous teeth and in first permanent molars as these are the teeth that start to form before birth. The neonatal line

was shown by Schour to be present in 90% of his sample of 350 teeth. Schour added that this line was present in both the enamel and dentine when a ground section was prepared passing through the highest points of the EDJ, the point at which enamel formation and mineralisation begins. Massler et al. (1941:45) reported that the neonatal line can be observed in '*virtually all deciduous teeth if sections are carefully prepared in a sagittal plane*'. Whittaker and Richards observed the neonatal line in 100% of their sample of 52 teeth as did Eli et al. (1989) in their sample of 147 teeth. Sarnat and Schour (1942) stated that the neonatal line was found to be present in 98% of teeth observed, while Norén (1984) reported it as being present in 93% of specimens examined in dry polarised light. Sabel et al. (2008) found that under polarised light the neonatal line was present in all of their exfoliated specimens (35 teeth), however, in their tooth bud specimens it was only observed in 95% of their sample and when observed in microradiographs the line was observed in 63% of this latter sample. This variation in the occurrence of the neonatal line using different observation techniques was reported several times by Norén; in 1978 Norén et al. (1978a), observed neonatal lines in 94-100% of their histology sections but only in 50-58% in their enamel microradiographs. While in 1983, Norén (1983) observed neonatal lines in 88% of his sample in dry polarised light and in 61% of his sample in microradiographs. Both Norén (1978a) and Sabel et al. (2008) stated that thin neonatal lines were more frequently observed in ground sections than in microradiographs and Sabel et al. suggested that this was indicative of changes in mineral density in the line and that a decrease in mineral content would not be revealed in a microradiograph. However, another possible reason for this might be that if a ground section is not cut perpendicular, then the line will appear thinner under light microscopy, which has a limited focus depth, while microradiographs which are not as sensitive for changes in the enamel quality will reveal a wider appearance of the neonatal line. Using scanning electron microscopy Sabel et al. (2008:958) observed the neonatal line at low magnification (x200) in all of their sample, however at higher magnifications they reported that the line became less distinct, although it could sometimes be recognised by a '*disturbance in the enamel organisation*'.

Eli et al. (1989:222) stated that even though they observed 100% of the neonatal lines in their sample, the line was continuous in only 60-78% of their sections, they suggested that the lower percentage of the neonatal lines observed in other studies may be '*due to the lack of appearance of the neonatal line in some areas of the tooth crown*'. Partial neonatal lines were also observed by Jakobsen (1974:101) in several of his sections; he suggested that the direction of the prisms in the section plane may be one reason why the neonatal line did not stand out as a complete demarcation of the prenatal enamel or that '*a difference in ameloblast sensitivity towards the birth trauma*' could be responsible for the partial lines that he observed. Although Gustafson and Gustafson (1967:97) reported that the neonatal line was sometimes '*very insignificant*', Skinner and Dupras (1993:1384) suggested that the lack of a neonatal line in some sectioned teeth could probably be attributed to '*vagaries in lighting and sectioning rather than actual absence*'.

Interestingly the incidence of the occurrence of the neonatal line in the first permanent molar was suggested as a method of sex determination by Jakobsen (1974). From a sample of all four first permanent molars from 16 males and 11 females, aged from five years to 45 years, Jakobsen discovered that the frequencies of the occurrence of the neonatal line in males and females, proved to be significantly different. Seven out of the 16 males had no neonatal lines at all in any of their four molars, while all 11 females exhibited a neonatal line in at least one first molar. The frequency of the occurrence of the neonatal line was higher in the females than in the males. This significant difference was found between the male and female groups as a whole, as well as between single teeth and single cusps. This investigation also demonstrated an even distribution of the occurrence of the neonatal line over all four jaw quadrants, with no suggested differences in maturation occurring between different sides or between jaws.

These findings seem to indicate that on average, males are less dentally mature at birth than females, with the absence of any neonatal lines in first permanent molars seeming to indicate that the tooth came from a male. This, Jakobsen

suggested, may be useful for the determination of sex in forensic investigations where skeletal remains are involved. However, this can only be a cautious assessment as Jakobsen reported that statistical calculations made on the basis of this material showed, that in larger samples some females may also lack neonatal lines. Additionally, the gestation age at the time of birth was unknown in this material, so it could be argued that the results obtained could have been influenced by the premature birth of some of the male specimens. However, on the basis of the differences found in the frequency of the occurrence of the neonatal line, it seems reasonable to suggest that skeletal remains are male in those cases where no neonatal line can be found in any of the four first deciduous molars.

The neonatal line is directly related to the time of birth. Its position in enamel appears to be consistent from the EDJ in each tooth type. It was this apparent consistency and characteristic position that led Schour (1936a:1947) to suggest that this particular line was caused by some '*constant and universal condition*'. Any variation in the position of the neonatal line depends on which tooth is being observed and the length of gestation. Although Schour initially described the line as occurring at a constant level within a tooth he later suggested that prematurity would shift the neonatal line occlusally up the enamel-dentine junction (Kronfeld and Schour 1939; Schour and Kronfeld 1938). This is a reasonable suggestion as the earlier the birth occurs the nearer the neonatal line will be to the enamel-dentine junction; likewise the later the birth occurs the more tooth crown is completed before birth and so the neonatal line is closer to the cervix of the tooth. As birth timing is variable, the location of the neonatal line should vary characteristically with gestation length, Skinner and Dupras (1993) found that gestation length did in fact correlate with the position of the neonatal line. Skinner and Dupras (1993) examined 173 ground sections of deciduous teeth from pre-term, term and post-term births using normal and polarised light at x20 magnification. They discovered that 73% of the neonatal lines that lie beyond 2SD of the mean location of the line in term births are from children born outside of 38 to 42 weeks gestation. The duration of pregnancy was found to account for 36% of the variation of the location of the neonatal line in non-term births. The remaining variations Skinner and Dupras (1993)

suggested could be attributed to differences in tooth size, individual variation and errors in estimation of birth timing, with reporting error being the most likely cause.

Although the analysis of variance failed to show statistically significant differences between the sexes it did confirm that the neonatal line was differentially located in each tooth type and that this varied significantly as a function of gestation length. Skinner and Dupras (1993:1386) identified that the '*normal location*' of the neonatal line '*can appear to depart by more than 9 weeks from its average position*'. However, based on the small proportion of non-term births whose neonatal line is located beyond 2SD of the mean location of the neonatal line in term births, Skinner and Dupras (1993) suggested that this technique will be able to contribute to the individualisation and identification of human remains in about 3-4% of immature skeletal remains in a forensic context.

The eruption times of deciduous teeth vary considerably, however, Szpringer-Nodzak (1984) investigated the position of the neonatal line in relation to the start of eruption. She examined ground sections from 379 central incisors from 311 children. By evaluating the position of the neonatal line she identified a correlation between the location of the neonatal line in enamel of deciduous incisor teeth and the time of eruption. It was observed that in teeth erupting later than 'normal' the neonatal line is located in the earlier formed enamel nearer to the EDJ, indicating either a later onset of tooth formation or a shorter gestation time. Likewise in teeth erupting earlier than 'normal', the neonatal line is located in the later formed enamel nearer the cervix of the tooth, indicating either an earlier onset of tooth formation or a longer gestation time. Szpringer-Nodzak (1984) suggested that the rate of dental development in the prenatal period influences the eruption time. The neonatal line in the teeth that had erupted on time but were from children who had been delivered prematurely, were '*distinctly visible, longer and located in the earlier formed enamel*' nearer the enamel-dentine junction (1984:4). This finding supports Schour's suggestion that prematurity would shift the neonatal line occlusally up the enamel-dentine

junction (Kronfeld and Schour 1939; Schour and Kronfeld 1938). So it appears that the position of the neonatal line varies with the length of gestation and the time of eruption.

Rushton (1933:170), originally reported that the contour lines he had been investigating under a low magnification were '*practically no thickness*', while Weber and Eisenmann (1971) reported the neonatal line to be about 20-30µm, Whittaker and Richards (1978) reported it as being about 15-16µm thick and Sabel et al. (2008) stated that the width of the neonatal line varied from 10-20µm. While Mahoney (2011) reported the neonatal line as being 10-25µm wide and forming over three to eight days. So from the literature it appears that the neonatal line can range from 10-30µm. However, Jakobsen (1974:99) reported that the width of the neonatal line '*is highly dependent upon the distance of the section from the cusp centre*', with increasing distance the neonatal line becomes wider and ends up '*completely blurred when the prenatal enamel is hit tangentially*'.

It has also been suggested that the width of the neonatal line may be related to the time it takes for the infant to regain weight after the trauma of birth. Jakobsen (1974:103) reported that the neonatal line was '*generally about 10µm*' wide and he suggested that '*although this is no exact indication of the duration of the disturbance of amelogenesis. It is considered reasonable to conclude that the neonatal line represents the same period of time which the new born uses to regain its birth weight*'. Norén (1983) investigated this matter further; using polarized light and microradiography he examined ground sections from 64 infants with a birth weight below 2000g and sections from 43 healthy infants. Norén (1983:360) stated that '*the more marked width of the neonatal line found among the low-birth-weight infants may be related to the severity and duration of the initial weight loss after birth*'. Norén (1983:360) suggested that there was an eight to ten week difference in gestational age between normal full term infants and the low-birth-weight-infants. He also stated that the position of the neonatal line varied with gestational age and that '*the shorter the gestational age the greater was the tendency for the neonatal line to be positioned towards*

the incisal parts of the tooth', further supporting the findings of Skinner and Dupras (1993) and Szpringer-Nodzak (1984). Unfortunately, however Norén (1983) does not supply any quantitative data with his work.

Norén (1984:155) a year later, described finding '*widened neonatal lines*' in children born to diabetic mothers, as well as various other hypoplastic subsurface defects in the postnatal enamel. He had examined ground sections from the teeth of 30 infants of diabetic mothers using polarized light and microradiography. Norén (1984:153) suggested that infants of diabetic mothers are more immature than their gestational age would indicate and that their '*calcium phosphate homeostasis is impaired in the same manner as in premature infants with a marked tendency to develop hypocalcemia and hyperphosphatemia*'. Norén (1984:153) concluded that the wider neonatal line in these teeth was related to the '*more frequent and more pronounced neonatal hypocalcemia occurring among infants of diabetic mothers*'. Again, unfortunately Norén (1984) does not supply any quantitative data with his work.

Massler et al. (1941:61) stated that the neonatal line is present at a characteristic level even in the teeth of children born by caesarean section. They suggested that this indicated that the neonatal line '*results from the changes in environment rather than from the birth type*'. However, it has since been demonstrated by Eli et al. (1989) that the width of the neonatal line may be indicative of the severity of parturition. Schour and Kronfeld (1938:473) had also previously stated that '*it is obvious that the greater the disturbance at birth, the more accentuated*' the neonatal line will be. The average width of the neonatal line in ground serial sections from the deciduous teeth of 147 children was measured by Eli et al. (1989) and this was then compared to the birth history of the child. In children with normal birth histories the mean width of the neonatal line was found to be between 11.9 and 12.4µm, while in children born by difficult operative delivery the line was wider (18.6+/-5.7µm) and in children born by elective caesarean section the line was thinner (7.6+/-1.5µm).

Although the 12µm mean neonatal line width obtained by Eli et al. (1989), is in near agreement with that obtained by Jakobsen (1974) (~10µm). Jakobsen (1974:99), had previously claimed that the width of the neonatal line '*is highly dependent upon the distance of the section from the cusp centre*', Eli et al. (1989) however disagreed, they stated that the level at which the sample was taken (incisal, middle or gingival) had little influence on the resultant value. They added that as the neonatal line is created by the trauma caused to the baby during or immediately after the birth process that '*this trauma simultaneously affects all ameloblasts at the different tooth levels establishing a basically uniform line*' (Eli et al. 1989:222). The fact that the width of the neonatal line increases significantly in children born by operative delivery and decreases in children who have undergone no active birth process, suggests that the change from the intrauterine to the extrauterine environment is responsible for only part of the arrest of the ameloblast function and that the trauma of the birth process itself has a major impact on the new born baby.

Eli et al. (1989:222) suggested that if the width of the neonatal line of children born by caesarean section is taken as '*indicating the effect on the ameloblast of the transition from intra- to extrauterine life (without active birth process)*' then this transition accounts for 63% of the neonatal line width. They proceeded to suggest that if the width of the 'normal' neonatal line is taken as 12µm, then this 12µm is partly caused by the environmental shock to the newborn (about 63%) and partly by the birth process itself (about 37%), and that this knowledge may be of considerable value when investigating causes of various pathological conditions. Eli et al.(1989) concluded that the birth process itself as well as the dramatic change from the intrauterine to extrauterine environment also contributes to the width of the neonatal line.

The extent of these transitional effects on the ameloblasts may vary under different environmental conditions. Schour and Kronfeld (1938:473) suggested that the neonatal line would be more prominent in the teeth of prematurely born children, due to the '*relatively greater nutritional difficulties and other disturbances usually encountered by premature infants*'. This may explain the

greatly increased neonatal line widths (20-30µm), reported by Weber and Eisenmann (1971) as their specimens were taken from prematurely delivered children.

It also appears that Massler et al.'s (1941:61) previous statement that the neonatal line '*results from the changes in environment rather than from the birth type*', needs to be amended to include the fact that the birth type does appear to influence the neonatal line.

4.3.2 Conclusion

As mentioned at the start of this section, '*birth is the most profound change in environment and nutrition which man experiences from conception to death*' (Kronfeld and Schour 1939:20; Schour and Kronfeld 1938:471). Therefore it may be reasonable to expect that the process of birth and the subsequent neonatal adjustment period will result in changes to many of the organs and tissues of the body. No matter how marked these changes may have been at birth, they soon become indistinct and finally become completely obliterated by the continuous process of growth and replacement to which most tissues are subjected, for example, the foramen ovale in the heart or the umbilical vein in the liver. However, teeth are an exception to this rule and changes that may occur during the formation and mineralisation of the enamel and dentine '*remain permanently engraved on these structures*' (Kronfeld and Schour 1939:20; Schour and Kronfeld 1938:471). These changes can be identified regardless of whether the tooth is still in the mouth, or whether it has been shed or extracted and this is one main reason that the developing dentition is of so much interest to the forensic anthropologist, osteologist, odontologist and evolutionary scientist.

The idea that birth with its associated nutritional and metabolic disturbances may leave a permanent mark on the teeth was first discussed in 1933 by Rushton. There are several possible factors that could contribute to the

occurrence of the neonatal line; the first of these is the shock of birth itself, it seems reasonable to suggest that passage of the fetal head through the birth canal, with increased pressure due to molding of the cranial bones, produces a marked upset in the metabolic processes of the infant, so that its growth for a short period is essentially slowed. The loss in weight of the newborn as mentioned earlier, may be caused in part by disturbances of metabolic processes incident to the shock of birth, as well as the disconnection from the maternal blood supply as the source of fluids and nutrients. It is very possible that the neonatal loss of maternal fluids and nutrients and the transfer of function to the neonatal gastrointestinal tract may be the principal factors in the interruption of growth which immediately follows birth. Another fact that may contribute to the formation of the neonatal line is the endocrine readjustment which occurs at birth when the mother's endocrine system ceases to be a factor in the endocrine balance of the infant. As discussed below, (see **Section 4.4**) the function of the endocrine system and particularly the role of the parathyroid glands has been shown to have a major affect on enamel development (Schour 1936b; Schour et al. 1937; Schour and Rogoff 1936; Schour and Van Dyke 1932).

Blood calcium levels in the neonate are higher than that of the mother due to active transport over the placenta through the action of PTHrP (parathyroid hormone-related protein), which is secreted by the parathyroid glands and which regulates the fetal calcium gradient (Hsu and Levine 2004). This fact also supports the expectation that prenatal enamel would be more highly mineralised than postnatal enamel, (see **Section 4.5**). Calcium levels in the neonate continue to rise, along with a rise in vitamin D and calcitonin, during the third trimester (Kovacs 2001; Salle et al. 2000).

At birth there is an abrupt disconnection from the maternal calcium supply and the newborn infant becomes entirely dependent on its own dietary calcium and skeletal calcium reserves. This disconnection results in a fall in serum calcium concentrations over the first twenty-four hours and a rise in PTH (parathyroid hormone) secretion in response to this. Low blood calcium levels, therefore, may well contribute to the formation of the neonatal line, as demonstrated in

rabbits by Okada (1943), in addition the fact that the neonatal line is radiolucent in microradiographs, confirms that there is a short period of hypomineralisation just after birth. This hypocalcemia continues for some days after birth as the infant's parathyroid glands do not respond very efficiently during the first two weeks of life and so there is a period of physiological hypocalcaemia while these glands begin to function normally (Hsu and Levine 2004). Salle et al. (2000) stated that calcium concentrations usually return to normal by days five to ten. This period may account for the wider diffuse zone that has often been observed occurring after the neonatal line (Gustafson and Gustafson 1967; Silness 1969; Whittaker and Richards 1978). Whittaker and Richards (1978:47) stated that '*it seems clear that some disturbance in physiological activity of the ameloblasts occurs at the time of birth and continues to a diminished degree in the succeeding few days*' and it is possible that this diffuse zone could be explained by the parathyroid glands establishing their normal function. Furthermore the ability of the parathyroid glands to respond to decreased calcium levels following birth is dependent on gestational age (Hsu and Levine 2004; Tsang et al. 1973) and this may also explain the more pronounced lines observed in premature births (Schour and Kronfeld 1938).

In addition infants born to diabetic mothers have even more severe hypocalcemia than that usually associated with parturition for which IGF1 (Insulin-like growth factor 1) and insulin levels have been implicated (Hsu and Levine 2004; Salle et al. 2000; Tsang et al. 1973). Thus hormones and growth factors other than those normally associated with calcium metabolism may have a significant impact on neonatal line formation, for example Norén (1984) and Norén et al. (1978b) reported that children of diabetic mothers do indeed have wider neonatal lines.

This 'birth line', 'neonatal line' or 'neonatal ring' in enamel and dentine was firmly established as having a definite neonatal origin on the following basis:

1) the recognition that the surface of enamel and dentine in the deciduous teeth of full term stillborn infants corresponds with the position and level of the neonatal line in older infants (Rushton 1933; Schour 1936a).

2) the observations in the enamel and dentine of an infant which at known intervals had received injections of a substance which resulted in pronounced striae in the tissues forming and mineralising at the time of injection (Schour 1936a; Schour and Poncher 1937).

3) the recognition that human enamel and dentine grow at an average daily rate of 4µm, with a growth gradient that is characteristic and constant for any particular region of a given tooth type (Schour 1936a; Schour and Massler 1937; Schour and Poncher 1937).

It was the identification of the neonatal line and the production of quantitative proof that this line was definitely related to the event of birth that lead to improvements in the development of chronologies of the deciduous dentition and into studies of pre- and postnatal enamel and dentine formation rates. Interestingly, Weber and Eisenmann (1971) using phase microscopic and microradiographic observation techniques, found no indication of the formation of the neonatal line in one of their specimens who lived for half a day or in another who lived for two days. Although its general association with birth is well established, the exact time at which the line is formed or the period during which it is forming is not known and indeed, may vary.

4.4 Neonatal Hypoplasia and Hypomineralisation

For every incremental line in the enamel there exists a corresponding line in the dentine and this can be clearly observed in longitudinal ground sections of deciduous teeth, most sections will show the neonatal line, which is a pronounced stria of Retzius in the enamel and a corresponding Andresen line in the dentine, both of which indicate the time of birth. Likewise for every incremental defect in the enamel there exists a corresponding defect in the dentine. However, although both dentine and enamel respond to mild disturbances in metabolism by a deficient mineralisation of the incremental layers forming at the time, their reaction to more severe systemic disturbances

is quite different. Enamel is more sensitive to systemic disturbances than dentine is and in severe systemic disturbances, enamel not only fails to mineralise properly, but often fails to form at all. The result is a hypoplastic defect in the enamel. Dentine however, is more resistant and often shows only a corresponding deficiency in mineralisation, the lack of or arrest of dentine formation is relatively rare.

Enamel hypoplasia is the deficient or arrested formation of enamel and hypoplastic enamel is the result of any condition that inhibits enamel formation during the secretion stage of amelogenesis. Any interference during this stage of amelogenesis leads to a reduction in the quantity and/or the composition of the enamel matrix, the resulting enamel is thinner than normal, however its density is generally normal and it is usually fully mineralised. Hypoplastic enamel may have a more yellowish or greyish hue to it and it usually manifests at different levels in the fully formed crown as pits or grooves on the enamel surface. This missing enamel may be localized, forming one small pit, or it may be completely absent altogether (Garant 2003; Stein 1947). A range of neonatal hypoplastic defects were described by Kronfeld and Schour (1939) and these are discussed below.

Enamel hypomineralisation is caused by any condition that inhibits enamel mineralisation during the maturation stage of amelogenesis; during this stage the hydroxyapatite crystallites expand in size until they are tightly packed together. If the crystallites do not grow to full size, then they are less tightly packed and the enamel is not 96% inorganic. Under these conditions the enamel is said to be hypomineralised. Any interference during the maturation step of amelogenesis affects the ameloblasts, resulting in a reduction of the quality of maturation of the developing enamel. Enamel affected by hypomineralisation is usually of full thickness but more porous, less dense and less mineralised than normal and as a result it may decay more rapidly. In cases of hypomineralisation the enamel surface is generally intact but it tends to be more opaque rather than translucent (Garant 2003; Stein 1947).

Disturbances in mineralisation are much more common than disturbances in

formation and there is extensive experimental as well as clinical evidence to demonstrate this (see below). Furthermore, the same interference may cause either a hypomineralisation or a hypoplasia, depending on the severity of the disturbance. A mild disturbance may cause deficient mineralisation without affecting the formation of the enamel matrix, but a very severe disturbance may cause a hypoplastic defect in addition to deficient mineralisation. Thus, hypomineralised and hypoplastic defects, although arising from two different developmental processes, are related in as much as they indicate different degrees or intensities of disturbances. Such defects in enamel may be of local or systemic origin, the latter being far more common. Systemic hypoplasia follows a developmental pattern (unlike local hypoplasia which can occur as the result of an incident of infection), since the etiologic agent is of a systemic nature it therefore affects all the formative cells that are active at the same time; so chronologically, it affects all of the corresponding areas in the different teeth that are developing at that time.

As its name suggests, neonatal hypoplasia indicates a disturbance in the formation rather than mineralisation of the developing enamel and it originates during the neonatal period. In its mildest form neonatal disturbance is reflected as an accentuated stria of Retzius (neonatal line). In its most severe form neonatal disturbance can, as illustrated by Schour and Kronfeld (1938) and Kronfeld and Schour (1939), result in the complete arrest of enamel formation at birth or during the neonatal period, with postnatal enamel formation only occurring cervically up to the neonatal line. This severe neonatal hypoplasia results in a tooth with a very thin layer of prenatal enamel at the incisal or cuspal surface and a circular ledge of normal postnatal enamel around the cervical portion of the tooth.

As the crowns of the deciduous teeth develop partly before and partly after birth, the neonatal line plays a particularly important role in the analysis of hypoplasia of the deciduous dentition. Kronfeld and Schour (1939) suggested that if the frequency and severity of hypoplastic changes in the deciduous dentition are related to the difficulties and disturbances experienced at birth and

during the early postnatal period, then it would be reasonable to expect to observe more pronounced hypoplastic disturbances in children with a history of birth injury. As a result of this suggestion Kronfeld and Schour (1939) using 'tooth ring analysis' attempted to determine the times of occurrence of hypoplastic defects in three juveniles with a history of birth injury. For their first case they referred back to a case study that they had presented the previous year and which is discussed in **Section 3.4**, involving an infant who had sustained a brain injury at birth and from who they also developed their original deciduous chronology table (Schour and Kronfeld 1938:487). This individual exhibited a very severe form of neonatal hypoplasia, which had resulted in the complete arrest of enamel formation in some areas of the teeth. Kronfeld and Schour then presented two more case studies of individuals exhibiting hypoplastic defects, resulting from birth injuries and whose medical histories were also available, further illustrating that neonatal injuries could be observed in the deciduous dentition. The second case that Kronfeld and Schour presented described a seven year old girl who had been delivered prematurely and who had suffered an apparent birth injury, which had resulted in delayed physical development. Several of her deciduous teeth had been obtained when they had been exfoliated and these were prepared as ground sections. Kronfeld and Schour (1939:24) reported that these sections '*show very plainly the neonatal character of the hypoplasia*'. The tip of the crown was covered by a thin layer of prenatal enamel, on top of which and separated from it by a '*very pronounced neonatal line*' (1939:25) was a thick layer of postnatal enamel which ended short of the incisal edge, resulting in the clinical appearance of enamel hypoplasia. Similar to the first case study this enamel hypoplasia had a neonatal origin which was illustrated by the fact that up to the time of birth the enamel had formed normally and the neonatal line was reported as forming a '*sharp dividing line*' between the normal prenatal enamel and the hypoplastic postnatal enamel (1939:25). In the third case, which was not as severe as the first two, a ground section from another child with a history of a traumatic birth injury was presented. This showed normal uniform prenatal enamel and an '*accentuated*' enamel neonatal line with '*possibly…poorer calcification*' of the postnatal enamel (1939:25).

These three cases clearly illustrate that the presence of the neonatal line can be used to establish whether trauma to the ameloblasts is of a neonatal or postnatal origin and also that varying degrees of neonatal trauma are very clearly identifiable as hypoplastic defects in the deciduous teeth developing at the time the trauma was sustained.

Schour was involved in several experimental studies using animal models that demonstrated that developing teeth are very delicate structures and are able to record metabolic disturbances very accurately. Schour and van Dyke (1932) demonstrated this sensitivity in the enamel and dentine of the incisors of 23 hypophysectomised rats. Although the removal of the pituitary gland mainly influenced the eruption of teeth, areas of arrested enamel formation and hypoplastic regions were also reported and '*occasionally appearances similar to the bands of Retzius are seen*' (1932:419), no further details were provided regarding this latter observation. Schour and van Dyke (1932:417) suggested that as '*endocrine organs may be closely interrelated*' that by removing the pituitary gland they were also '*disturbing the parathyroids*', which are known to play an important role in calcium metabolism and the removal of which is known to disturb tooth development. However, as the incisor of the hypophysectomised rat presented with a different set symptoms from those of the parathyroidectomised rat, Schour and van Dyke (1932:417) added that the parathyroids are '*not playing a primary role*' and that they '*very likely play an incidental role*' in these changes.

Schour and Rogoff (1936) again demonstrated the sensitivity of developing teeth to metabolic disturbances, this time in the incisors of 45 rats following bilateral adrenalectomy and again '*disturbances in calcification*' (1936:343) were reported. Schour and Rogoff suggested that there was a possible functional interrelationship between the adrenal and parathyroid glands and that adrenal insufficiency like hypophyseal insufficiency is also associated with disturbances in calcium metabolism.

Schour (1936b) demonstrated cases of disturbed mineralisation in the incisors of 26 ground squirrels following a bilateral gonadectomy. From this research Schour (1936b:192) observed that the '*organic matrix persists to an abnormal extent*'. Schour (1936b:189) again suggested the possibility of a functional interrelationship existing between the endocrine glands and stated that the '*removal of the gonads results in particular disturbances in the other endocrines*' and that this insufficiency was also associated with disturbances in calcium metabolism.

In 1937, '*disturbances of calcification and growth*' were reported by Schour, Chandler and Tweedy (1937:954) following their observations of the incisors of 100 rats who had received parathyroidectomies. They also observed that the dentine that had formed and mineralised during and immediately after the operation showed a '*fine sharp line or sometimes a double line*' (1937:965). Schour et al. stated that a similar line was also observed in dentine subsequent to adrenalectomy (Schour and Rogoff 1936) and hypophysectomy (Schour and Van Dyke 1932) and which was located in the dentine that had been forming and mineralising at the time of these operations (these lines may be the Retzius type lines mentioned above, however no further details are provided). This acute response Schour et al. (1937:965) stated was '*an expression of a shock to calcium metabolism*', which may have been induced by the trauma incident to surgery, the ether anaesthetic that had been used or acute endocrine disturbance. Schour et al. (1934:332), suggested that this resultant line may be the product of an over mineralisation effect associated with a very brief period of arrested growth similar to that observed by Harris in bone and that this is a '*chronic reaction and indicative of an effort at healing*'. Regardless of the cause of this reaction, Schour et al. (1937:965) considered this line to be '*an experimentally or otherwise induced hypercalcification*' effect in the dentine and they referred to it as a '*calciotraumatic*' line.

Although the disturbances in mineralisation identified by these experimental studies are not identical, this work does suggest that there is a relationship between disturbances in mineralisation and pituitary (Schour and Van Dyke

1932) adrenal (Schour and Rogoff 1936) gonad (Schour 1936b) and parathyroid (Schour et al. 1937) dysfunction.

A similar acute response was also found to be associated with the effects of injections of parathyroid extract (Schour et al. 1934) and sodium fluoride (Schour and Poncher 1937; Schour and Smith 1934a and b). In nearly every one of these cases the post injection period demonstrated a '*primary hypocalcified stripe*' (Schour et al. 1934:329), which was characterised by incomplete or deficient mineralisation, the position of this line corresponded with the time immediately following the injection. This primary stripe was then followed by a '*secondary hypercalcified stripe*' (Schour et al. 1934:330), which was characterised by over mineralisation and which corresponded in its position with the time approximately subsequent to the first twenty-four hour post injection period (in rats). Interestingly, Schour et al. (1934:331), reported that an '*extensive series of rats which were treated with other tissue extracts showed no reaction*' in their developing dental tissues, this comment is unfortunately cited as 'unpublished data' and no further details could be located regarding this statement.

These experimental studies illustrate the extreme sensitivity of the developing teeth to disturbances in calcium metabolism. Erdheim (1911) according to Schour had previously pointed out that '*the dentin of the rat incisor acts like the drum of a kymograph, in which are recorded the disturbances in calcium metabolism in an accurate and easily readable manner*' (Schour 1936a:1954). This is a reference to a concept that appears frequently throughout Schour's work (Massler et al. 1941; Schour 1936a; Schour 1938; Schour and Kronfeld 1938; Schour and Massler 1940a; Schour and Poncher 1937). Schour added (1936a:1954) that the existence of the neonatal line in the enamel of human deciduous teeth '*confirms Erdheim's comparison and amplifies it*'. This analogy Schour suggested, is applicable not only to the dentine of a rat but that it also applies to both the dentine and enamel of the human tooth whilst they are forming and mineralising and also to other metabolic changes as well as fluctuations in calcium metabolism. The delicate nature of this enamel and

dentine reaction is demonstrated by the fact that in a number of Schour's sections he reported that the enamel that preceded the formation of the neonatal line differed markedly from the enamel that was formed subsequently. He added that in ground sections, the prenatal enamel appeared to be better mineralised than the postnatal enamel. The inference here being that prenatal mineralisation of enamel is often of a higher quality than postnatal mineralisation (see **Section 4.5**).

Enamel constitutes a permanent record of the systemic and local conditions which influence the ameloblasts during formation and mineralisation, with the resultant hypoplastic or hypocalcemic events illustrating this; such defects have therefore been reported as being a '*permanent record of nutritional disturbances and of diseases that occurred during the formative period of the teeth*' (Kronfeld and Schour 1939:18). As the developing tooth permanently records these normal and pathological variations in metabolism in its growing structure, this makes it possible to analyse these metabolic disturbances and this may assist in the assessment of health and disease of the individual. The time of the effect can be determined by the chronological position of the affected incremental layer. While the intensity of the condition will be reflected in the degree to which the particular incremental layer is deficient in either formation or mineralisation. Although it may not be possible to identify the specific cause of the effect, as enamel hypoplasia may be produced by a variety of causes (rickets, tetany, fluorosis and fevers as described elsewhere in this thesis), it is possible to establish the intensity and the timing of the hypoplasia, which will reflect the intensity and time of the cause.

4.5 Prenatal and Postnatal Enamel

One of the earliest attempts to distinguish histologically between prenatal and postnatal mineralised tissues was by Karnosh in 1926 during his analysis of syphilitic enamel hypoplasia. From ground sections of hypoplastic first permanent molars from patients with congenital syphilis (mulberry molars), Karnosh (1926:34) identified '*prenatal caps*' of enamel which he stated are

'*sharply defined and show little or no structural deficiencies*'. Immediately above these layers, are '*the broad lamellae representing the first year's calcification*' which show '*deeply stained enamel hypoplasia*'. Karnosh (1926:34-35) stated that there is a '*sharp contrast between the deposits made before birth and those immediately after*'. Interestingly, Karnosh (1926:34) mentioned that this hypoplasia is at its greatest intensity at the '*birth line*'. This mention of the 'birth line' predates both Rushton and Schour, unfortunately however, no further details are given or can be found regarding this comment and neither Rushton or Schour refer to this citation.

Karnosh (1926), Mellanby (1927) and Swanson (1931b) all agreed that mineralisation of prenatal enamel is better than that of postnatal enamel. Mellanby examined 1036 deciduous ground sections and found that 85.6% contained structural defects. She found that the second molars were the worst mineralised teeth, while the incisors were the best mineralised teeth with the maxillary teeth being slightly more defective than the mandibular teeth. Mellanby (1927:747) stated that the '*regions in which defects are seen most commonly*' seemed to indicate that '*before birth there is much less chance of interference with the calcification processes than after birth*'. She suggested that this is why the earlier formed teeth are comparatively well mineralised when compared to the teeth that are formed later.

Swanson (1931b:2176) stated '*good conditions for enamelization must prevail in early fetal life*' and he added that '*calcification must take place under increased difficulty as interuterine development proceeds*'. It appears that Swanson came to this conclusion by referring to the deciduous chronology produced by Pierce in 1884, who had stated that the deciduous teeth mineralise from the anterior teeth to the posterior teeth. Swanson then correlated this chronology with the findings of Mellanby (1927) who had reported in her work that this was also the order in which the severity of hypoplasia increased in deciduous teeth.

However, although these researchers are in agreement about the state of mineralisation of prenatal verses postnatal enamel, without the establishment of

the neonatal line it could be questioned that the identification of the enamel that they were observing was relatively imprecise. Kronfeld and Schour (1939) raised the point that without a definite landmark (neonatal line or a vital injection) researchers may not be able to distinguish clearly between prenatal and postnatal tissues. Furthermore, Massler et al. (1941) suggested that the chronological tables used by these authors were inaccurate. Kronfeld and Schour (1939) also suggested that one of Mellanby's 1927 figures actually illustrated neonatal hypoplasia and had been incorrectly identified as '*gross hypoplasia*' by Mellanby (1927:Fig 6). They had arrived at this conclusion due to the similarity between this figure and their own sections from infants with known neonatal injuries.

Confirmation regarding the state of prenatal and postnatal mineralisation therefore was not possible until the work of Rushton (1933) and Schour (1936a) which enabled researchers to clearly demarcate between prenatal and postnatal enamel. In 1936, following the identification of the neonatal line and the confirmation of its neonatal origin Schour reported that the enamel that preceded the formation of the neonatal line differed markedly from the enamel that was formed subsequently. Schour (1936a:1954) added that in ground sections, prenatal enamel appeared to be '*better calcified*' than postnatal enamel, he suggested that this indicated that the prenatal mineralisation of enamel is '*often better than postnatal*' mineralisation (Schour 1936a:1954).

Rushton (1939:2) commented that although '*contour-lines are occasionally seen in antenatal enamel*' structural defects are not as common as they are in the postnatal enamel. Using birefringence analysis (see **Section 4.3**) to observe pre- and postnatal enamel of 50 ground sections Rushton concluded that the mineralisation of the prenatal enamel is not homogenous and is not of a higher quality, except occasionally in isolated patches; furthermore there is often considerable variation in the degree of mineralisation throughout the prenatal enamel. He stated that although prenatal enamel lacks structural defects it is not very highly mineralised, while postnatal enamel is commonly more highly mineralised but has abundant structural defects. However, Massler et al.

(1941:59) suggested that this birefringence analysis method actually analysed the '*crystalline arrangement of the precipitated salts*' rather than the quality or density of mineralisation.

In 1939 Kronfeld and Schour (1939:25) examined the degree of mineralisation of prenatal enamel, using a sample of '*approximately fifty complete jaws of infants and young children*' as well as the '*shed or extracted deciduous teeth of more than 600 additional children*'. From this large sample they concluded that prenatally formed enamel does show '*uniformly good calcification*' (Kronfeld and Schour 1939:25). Using the neonatal line as a biological landmark to demarcate the prenatal and postnatal enamel, Kronfeld and Schour (1939) supported the statements made by Hess et al. (1932) and concluded that in utero, mineralisation normally takes place '*homogeneously and completely*' (1939:26) and that '*prenatal hypoplasia is extremely rare*' (1939:31). Hess et al. had stated that the total amount of mineral salts present in the teeth of newborn infants was too small to be influenced by the maternal diet. They had established that only 0.5gm of calcium phosphate is present in the teeth of both jaws at birth and they stated that this amount could be removed from the mother's skeleton '*without suffering the slightest harm*' and without the need to supplement her diet with additional calcium and phosphorus (Hess et al. 1932:1059). Hess et al. added that the effect of the prenatal mineral metabolism of the mother is, when compared to the infant's postnatal nutrition, of little significance to the infant's teeth. Kronfeld and Schour (1939) added that in utero the process of mineralisation is not normally influenced by fluctuations in the condition of the mother, as the fetus is so well protected by the uterus, except perhaps in cases of severe maternal illness or a deficiency, such as osteomalacia, (see below). After birth, however the state of mineralisation is quite different from that of prenatal enamel and mineralisation of the teeth is very much influenced by the infant's health. This influence can first be identified in the neonatal period as the appearance of the neonatal line in every deciduous tooth and first permanent molar. Then according to Kronfeld and Schour (1939:27) zones of disturbed mineralisation can usually be found in teeth '*particularly from birth to ten months of age, which express the vicissitudes of the first few years of life*'. This period of time from birth to ten months Massler

et al. (1941) defined as the 'infancy period' (see **Section 3.3.4.c**).

As mentioned above, '*prenatal hypoplasia is extremely rare*' (Kronfeld and Schour 1939:31). Although this does appear to be the case in the majority of the literature, there are a few reported exceptions. One of the first cases reported in the literature was by Maxwell (1930) who presented two cases of 'fetal rickets' in Chinese infants. In one of these cases a demineralised stained section of a developing incisor from a five day old baby was described as showing '*defective enamel structure*' (1930:Fig 1), while all of the teeth were reported as showing '*marked enamel hypoplasia*' (1930:330); the mother had suffered from prolonged untreated osteomalacia. In 1935 Wolfe described three cases of 'fetal rickets' again in Chinese infants, who displayed hypoplastic defects in their prenatal enamel. The mothers of these infants had suffered from gross nutritional deficiencies, as evidenced by the clinical observation of tetany, osteomalacia and very low blood calcium levels. In his first case study Wolfe (1935:908) observed ground sections and from these he concluded that '*the period at which the deficiency occurred in utero can be stated*', he proposed that it may be inferred that '*calcification began normally at the seventeenth week and proceeded until the twenty-third week*' (1935:907), from this time onwards however, mineralisation was extremely poor. Normal mineralisation evidently had then resumed during the first week of extra-uterine life, at which time the rachitic condition had been identified and appropriate treatment given. Wolfe (1935:908) reported the '*line separating the hypoplastic from the normal enamel*' as being '*sharp*' and he then proceeded to say that this sharp line was '*indicative of the rapid response of the enamel organ to the improvement in nutrition of the infant*'. Wolfe (1935:911) also examined demineralised and ground sections from two more cases, this time from stillborn infants and again observed a '*marked irregularity of calcification*' in the prenatal enamel.

In addition to these cases mentioned above, Massler et al. (1941) also observed three cases with prenatal hypoplastic defects. They concluded that these were probably the result of severe under nutrition of the mother during pregnancy.

Massler et al. (1941), investigated the density of mineralisation throughout enamel using transmitted light to help judge the degree of translucency and homogeneity of the enamel structure. Areas that appeared dark or black were regarded as poorly mineralised. The number of incremental lines was also used as another index of mineralisation, the greater their number the lesser the degree of mineralisation. Swanson (1931a:819) had previously recognised that the striae of Retzius '*range in a graded series from scarcely perceptible to relatively grave lesions, and reflect, by their magnitude and number, the changes that took place in the salt balance of the body as enamelization proceeded*'.

From their observations of ground and demineralised sections from about 1000 human deciduous and permanent teeth, from '*normal, healthy children*' which they stated were distributed evenly between deciduous and permanent teeth and between all tooth types, Massler et al. (1941:44) concluded that prenatal enamel is '*characteristically white and translucent. The calcification appears to be homogenous and dense, relatively few incremental bands being present*'. They did not find any hypoplastic defects in the prenatal enamel, although they stated that three cases of prenatal hypoplasia had been observed elsewhere (see above). In this study, the enamel neonatal line was reported as appearing as '*dark or hypocalcified*' (Massler et al. 1941:45) and it was suggested that it '*probably represents a line of arrested growth rather than a line of disturbed calcification*'. Only in a few cases did they observe hypoplastic defects in the neonatal line itself, one of these cases involved the infant who had sustained brain damage at birth and which has been mentioned elsewhere in this work.

The mineralisation of postnatal enamel was described as being '*definitely less homogenous than during the prenatal period*' (Massler et al. 1941:45). Massler et al. (1941:45) also reported that accentuated striae in the postnatal enamel are '*the rule rather than the exception*'. This decrease in the quality of enamel mineralisation is reported as being only slight during the first three months, but becoming increasingly more prominent from the third to the tenth month. At the

tenth month however, an abrupt recovery in the mineralisation quality was frequently observed. Massler et al. (1941:52) suggested that this recovery was due to the fact that by this age the child has a '*more independent existence; improved alimentation and antibody mechanism*'.

The occurrence of hypoplastic defects in the enamel was identified as being the highest during the postnatal period, with over 70% of the total of the hypoplastic defects occurring from birth to ten months, these were termed 'chronic hypoplastic defects', again these defects were reported as usually ending abruptly at ten months, which according to Massler et al. (1941:47) indicated '*a complete and sudden recovery from the systemic disturbance or a difference in the metabolic and cellular response*' of the enamel at that age. They justified this conclusion as their records showed '*no change in the clinical condition of the patient*' (Massler et al. 1941:47). Only 1% of the hypoplastic defects occurring during this period did not end at the tenth month, evidently these were so severe that they continued into the second year and beyond, affecting practically all the teeth at all levels. Only about 2-5% of the hypoplastic defects of the infancy period are reported as being acute and limited to the level of the neonatal line (hypoplasia of the neonatal line) or the 'infancy ring' (acute hypoplasia of the infancy ring').

In microradiographs of postnatal enamel Crabb (1959:119) observed '*radiopaque and radiolucent bands lying parallel to the striae of Retzius*'. He reported that in fetal material such lines appear to be the exception, however when they are present in prenatal enamel they are poorly defined. Crabb suggested that this finding supported the observations of Rushton, who had also stated that structural defects are more common in postnatal enamel than in prenatal enamel. However, Gustafson and Gustafson (1967) stated that although postnatal enamel often shows such disturbances, this is not always the case. They also reported that sometimes the postnatal enamel was as well mineralised as the prenatal enamel and sometimes it was even better mineralised than the prenatal enamel.

Allan (1959:1105) observed '*sharp variations in the degree of mineralisation*' of the pre- and postnatal enamel. He suggested that this association of the variations in mineralisation with the incremental pattern of the organic matrix indicated that the cause of this variation lies in '*differences in the composition of the organic matrix, and the effect of these differences on its ability to accept mineral matter*'.

Silness (1969:96) using microradiography and light microscopy observed that prenatal enamel was '*uniformly dark*' and was '*devoid of structural details*', while '*alternating light and dark lines*' extended through most of the postnatal enamel. Although more frequent in postnatal enamel, Silness also reported cases where '*linear variations in radiodensity*', were also present in prenatal enamel, however these lines were always more distinct in the postnatal enamel (1969:96). He also reported a gradual decrease in mineral content from the surface towards the radiolucent neonatal line, however, in several teeth, he also observed that the prenatal and postnatal enamel close to the region of the line seemed to be mineralised to the same degree and that the line did not actually appear to demarcate two different regions of mineralisation. Silness stated that the enamel formed at birth and the immediate postnatal enamel seemed to be particulary more susceptible to disturbances in mineralisation, than the prenatal enamel.

Using electron microscopy Weber and Eisenmann (1971) reported a fine granular material present in the space between the crystalline borders of the pre- and postnatal enamel, with no apparent difference between the morphology of the pre- and postnatal enamel crystals.

Both Jakobsen (1974) and Skinner and Dupras (1993) used the characteristic appearance of the pre- and postnatal enamel to positively identify the neonatal line. Jakobsen (1974:97) used the '*highly homogenous enamel layer closest to the dentin*' to establish the extent of the prenatal enamel, while the postnatal enamel he reported '*clearly differed from the prenatal enamel by exhibiting*

pronounced Retzius striations'. Skinner and Dupras (1993:1385) also used the differences between the '*characteristic homogenous*' prenatal enamel which appeared as a '*light color under polarized light and is usually free of even faint striae*', to establish the location of the division between the postnatal enamel which, '*often has faint striae and assumes a different hue under polarized light*'. These differences again being used to positively identify the neonatal line

In ground sections of the teeth of low-birth-weight infants Norén (1983) observed diffuse areas of increased porosity and distinct subsurface lesions in the negatively birefringent postnatal enamel; as well as increased hypoplasia along the length of the neonatal line. Norén also observed hypoplastic subsurface defects in the postnatal enamel of sections from children who had been born to diabetic mothers. He stated that postnatal enamel seemed to be more susceptible to disturbances in mineralisation than prenatal enamel (Norén 1983; Norén 1984). He also suggested that the enamel hypoplasia that he had observed was the result of severe neonatal hypocalcemia (see **Section 4.3.1**).

Wilson and Beynon (1989) used quantitative microradiography to further assess the findings of previous authors. They concluded that there were differences in the level of mineralisation between deciduous and permanent enamel with deciduous teeth being less well mineralised. Wilson and Beynon (1989) also identified a gradient of mineralisation that increased from the EDJ to the outer enamel in both permanent and deciduous teeth. They pointed out that the distance from the EDJ needs to be taken account of as the thinner enamel in deciduous teeth partly underlies this difference; they observed that deciduous teeth did not seem to have the higher mineralisation levels in prenatal enamel close to the EDJ as previously reported by other researchers. Interestingly, they also stated that there was a unique pattern of higher mineralisation at the cervix than the cuspal region in deciduous molars (the reverse of the permanent enamel and the deciduous incisors and canines) which they suggested was due largely to the lack of a gradient between the two regions in deciduous enamel.

Sabel et al. (2008) found that prenatal enamel appeared to be positively birefringent and that postnatal enamel appeared to be negatively birefringent with a well mineralised enamel surface. Using scanning electron microscopy Sabel et al. (2008:958) observed that the prisms in the prenatal enamel where '*slightly more irregular, indicating that prenatal enamel is not fully mineralised*', while the postnatal prisms had a more '*regular pattern*'. These findings are in concurrence with their polarised light microscopy observations. Prenatal and postnatal prisms were also observed to have different diameters, with the mean prenatal prism being 5.35µm and the postnatal prism being thinner at 4.80µm, a difference of 0.55µm. Sabel et al. (2008:962) suggested that this may '*reflect a not yet fully mineralised prism*' or it may '*reflect a consisting modification of the ameloblast at the neonatal line, resulting in a smaller diameter of the prisms*' in the postnatal enamel.

Several suggestions have been put forward regarding the reason why there is such a difference between pre- and postnatal enamel. Mellanby (1927) suggested that the reason for the decrease in the quality of mineralisation was due to an increase in the rate of enamel formation. Massler et al. (1941:62) disagreed, stating that '*the most rapid rate of growth of enamel and dentin, as well as the best calcification, occurs prenatally*'. Although this is partially correct, Massler et al. (1941:62) also added that the rate of formation of enamel '*follows definite laws of growth gradients*' whereas the mineralisation pattern proceeded irrespective of these rates and gradients of growth. Swanson (1931b) attempted to correlate the periods of good and bad mineralisation with the slow and rapid rates of body growth. He concluded that a relationship did exist and that there was a correlation between slow body growth with good mineralisation and fast body growth with poor mineralisation. Although the rate of growth was more rapid in the early months after birth and according to Swanson this would be more disadvantageous to the degree of mineralisation of the developing enamel, Mellanby (1927) stated that during these early months a diet of milk whether human, cow or artificial, is highly compatible with the formation of well-mineralised teeth. However, as the baby is weaned and its milk intake is decreased Mellanby suggested that these conditions are now compatible with defective mineralisation in enamel, which explains the decrease in mineralisation quality after birth. Essentially, the argument fits with the scenario

that during the transition from breast or bottle feeding to the intake of supplementary foods, systemic upsets become more numerous and therefore enamel mineralisation is prone to increased disturbance.

Massler et al. (1941:52) suggested that prenatal enamel exhibits good mineralisation and that the rare occurrence of hypoplastic defects compared to the postnatal enamel is because the fetus leads a '*parasitic existence*' and is so well protected by the uterus. The fact that enamel formed during the first three postnatal months shows relatively better mineralisation than that formed during the subsequent six months is due, Massler et al. (1941:62) argued, to the fact that '*fetal mineral reserves have not yet been exhausted*'.

Interestingly Massler et al. (1941) also discussed their findings regarding the social class from which the children who donated their teeth originated. Mellanby (1927:745) had previously reported that she had observed differences in the mineralisation of teeth obtained from private sources and from dental clinics, with the private cases being '*generally better formed*'. Massler et al. (1941:47) found that in patients from the '*well-to-do classes*' although the quality of mineralisation was still poorer in postnatal enamel than it was in the prenatal enamel it was on the whole better than the postnatal enamel from patients from '*the poorer sections*'. In this latter group more accentuated striae were also reported as being present.

4.5.1 Conclusion

Prenatal enamel exhibits '*almost perfect calcification*' (Massler et al. 1941:59). This is not really surprising when the environment of the developing enamel is considered. The fetus develops in an extremely well protected and optimal environment, existing as a parasite by deriving all of its nourishment from its mother and drawing on her calcium reserves when necessary.

Many of the above observations regarding the level of 'calcification' or

‘mineralisation’ may actually be references to the number of accentuated striae present or areas of disturbed enamel formation, rather than the degree of hardness or mineral content. Earlier studies did not quantify the degree of mineral content and the ‘quality’ of enamel formed was assumed to be the same as ‘degree of mineralisation’. In fact, the relative lack of accentuated striae prior to birth and the relatively larger number that were present from about six months onwards as infants are introduced to supplementary foods (especially in teeth from individuals from nutritionally deprived environments), suggests it is this that underlies many of the earlier assumptions that have been made regarding pre- and postnatal enamel formation.

The neonatal line has been reported as being the result of ‘*the brief arrest in growth subsequent to birth and reflects the physiologic readjustments incident to birth*’ (Massler et al. 1941:60). Again this is not really surprising when the severe metabolic disturbances which the newborn experiences as it leaves the optimal conditions of the uterus and enters the relatively less favourable extrauterine environment are considered. Massler at al. (1941:47) stated that 70% of all hypoplastic defects have their inception at birth and then continue through infancy (two weeks to ten months after birth). The reason for the increase in postnatal hypoplastic defects suggested by Massler et al. (1941:60) is due to the ‘*large number of pathologic conditions which constantly arise as a result of the trauma of birth*’. Pathological accentuations of the neonatal line depend on the severity of the adjustments during the neonatal period and have been observed in the cases of birth injuries (Kronfeld and Schour 1939; Schour and Kronfeld 1938), low-birth weight infants (Norén 1983) babies born to diabetic mothers (Norén 1984) and cases of difficult operative deliveries (Eli et al. 1989). These accentuations can appear as wider lines, deviations in the prism path of ameloblast activity, or as hypoplastic defects. Birth injuries occurring in premature infants are also particularly likely to result in hypoplastic defects in the neonatal line (Massler et al. 1941). In fact it seems that most trauma experienced at birth appears to accentuate the neonatal line in proportion to its severity.

Postnatal enamel contains more structural defects than prenatal enamel and as mentioned above, 70% of these hypoplastic defects are instigated at birth, Massler et al. (1941:61) termed this '*chronic hypoplasia of infancy*'. Postnatal mineralisation has also been reported as being '*relatively poorer during the first ten months of life*' (Massler et al. 1941:61). Again this increase in postnatal hypoplastic defects and poor mineralisation is not really surprising when the environment of the developing enamel is considered. This is a period of major transitional change, in which the organs and structures of the fetal body have to adapt and adjust to the more complex modes of growth that occur during childhood. Massler et al. (1941:61) suggested that the '*first ten months of life is a period during which the metabolism and cellular activities are highly susceptible to constitutional disturbances*'*.* The additional, strain of the rapid body growth which characterises the early developmental periods and a change in diet may also contribute to the poor level of postnatal mineralisation.

Throughout the formation of the deciduous enamel crown the infant undergoes developmental changes which involve a complete and traumatic change in metabolism as well as other cellular and constitutional processes; it is therefore not surprising to find such changes reflected in the enamel developing at that time.

CHAPTER 5: Materials, Methods and Data Collection

5.1 Materials

An initial sample consisting of 219 ground sections made from modern exfoliated deciduous central incisors, lateral incisors, canines, first and second molars were selected that exhibited minimal occlusal and lateral wear. Mandibular teeth were selected as these tend to be found as individual skeletal elements, in both an archaeological and forensic context; while maxillary teeth tend to remain in situ in the maxilla enabling other methods of age estimation such as the degree of dental eruption or suture closure etc. to be used in these situations. Ground sections with minimal wear were selected in order to observe as much of the enamel thickness (prism length) as possible while sections showing evidence of trauma, or pathology such as caries were discarded from the sample.

From this sample of 219 ground sections, 109 were prepared by the author, through the true longitudinal buccolingual plane using the method described by Dean and Beynon (1991), although the embedding stage was omitted and instead the crown was coated with cyanoacrylate cement (‘Superglue’) before sectioning. In the case of the deciduous molars, the plane of section was through the two mesial cusps (protoconid and metaconid). From these ground sections, further sections were selected that showed clear neonatal lines and clearly visible daily cross-striations in transmitted polarised light. As the clarity of cross-striations varies from tooth to tooth and can even vary throughout the crown of a single tooth, a diagram of each ground section was produced using a Wild Heerbrugg light microscope and a drawing tube at an objective magnification of x12 and eyepiece of x10. These preliminary drawing tube illustrations were used as the basis for selecting two further sub-samples from the large initial sample. Transmitted polarised light was used to help identify the regions of the clearest cross-striations these areas were then marked onto the diagram along with the position of the neonatal line. These illustrations

facilitated the selection of the most suitable ground sections for the production of photomontages.

The quality of this initial sample of ground sections, where regions of clear cross-striations and good neonatal lines were visible, was further improved by the inclusion of ground sections from dental teaching collections previously prepared by Dr Don Reid and Dr Helen Liversidge and another collection prepared by Professor Christopher Dean.

5.1.1 Sample Selection

From the large initial sample (n=219), two samples of ground sections were finally generated; the first group (sample group one) consisted of twenty sections and the second (sample group two) consisted of fifty sections (see **Table 5.1**).

Table 5.1: This table shows the initial sample size and the number of ground sections selected from each collection to form the final two sample groups.

		Number of Sections Selected	
Origin of Collection	**Collection Size**	**Sample Group One**	**Sample Group Two**
Dr Don Reid	92	5	11
Prof Christopher Dean	11	2	7
Dr Helen Liversidge	7	2	4
Wendy Birch	109	11	28
Total	**219**	**20**	**50**

5.1.2 Sample Group One

Four ground sections of each tooth type (20 in total) were selected for the first and third series of analysis (**Sections 5.3.1** and **5.3.3** below).

Using the preliminary survey drawing tube illustrations, the height of the crown was divided into three equal portions from the dentine horn along the EDJ to the cervix of the crown. These portions were designated as the ‘occlusal’, ‘lateral’ and ‘cervical’ regions of enamel (see **Figure 5.1**). Ground sections were selected where daily cross-striations were clearly visible in transmitted polarized

light and could be tracked running along the prism paths between the EDJ and the enamel surface throughout each of these three designated regions on both the labial/buccal and lingual aspects for each tooth type.

From these 20 ground sections, five had been produced by Dr Don Reid and were from a dental teaching collection from Newcastle-on-Tyne dental school, two had been produced by Dr Helen Liversidge (one from the Spitalfields collection the other from a dental teaching collection from the Royal London dental school), two had been produced by Professor Christopher Dean and 11 by the author from the juvenile population around University College London.

5.1.3 Sample Group Two

Ten ground sections of each tooth type (50 in total) were selected for the second series of analysis (**Section 5.3.2** below).

Using the preliminary survey drawing tube illustrations the ground sections exhibiting the least worn crowns and the clearest, least oblique and most complete neonatal lines were selected. Care was taken to select ground sections where the neonatal line could be clearly identified on both the labial/buccal and lingual aspects of each tooth type.

Of these 50 ground sections, 11 had been produced by Dr Don Reid and were from a dental teaching collection from Newcastle-on-Tyne dental school, four had been produced by Dr Helen Liversidge (two from the Spitalfields collection and the other two from a dental teaching collection from the Royal London dental school), seven had been produced by Professor Christopher Dean and 28 by the author from the juvenile population around University College London.

5.2 Methods

5.2.1 Production of Photomontages - Sample Group One

As cross-striations are often difficult to count directly through the microscope, photomontages were produced to help with this procedure[9]. These montages were constructed from a series of overlapping photographic prints taken with an Olympus OM-2N camera loaded with Kodak Gold 200 film attached to a Carl Zeiss Janamed 2 light microscope with an apochromat 25x/0.65 ∞/0.17-A objective lens; from a 5 x 7 inch print of the negative the resultant field width was 410µm.

A sequence of photographs was taken along the course of the enamel prisms from the EDJ to the surface of the tooth, so that a complete record of the incremental growth of the enamel could be obtained. This record covers the first enamel formed next to the EDJ to the last layers of enamel formed at the tooth surface, i.e. that formed just before the tooth was exfoliated, avulsed or just before the death of the individual. Photomontages were constructed of the mid-occlusal, mid-lateral and mid-cervical prism tracks on both the labial/buccal and lingual aspects of each tooth type, resulting in six photomontages for each ground section (see **Figure 5.1**). This process was repeated for four ground sections of each tooth type, resulting in the construction of 120 photomontages.

[9] A photomontage is a continuous series of overlapping photographic prints consisting of small areas of an object which are then reconstructed to produce a larger representation of the object. In this case, small areas of enamel were photographed at a time with the use of a light microscope. The resultant prints were then carefully reconstructed to produce a larger representation of the section at an increased magnification. Although this is a lengthy procedure, this method has the advantage that it produces a hard copy of the data that can be manually marked to provide a permanent record of the cross-striation counts and which can easily be referred to at a later date if necessary. Several photomontages can be compared at the same time, which is difficult or impossible with other forms of digital image archives.

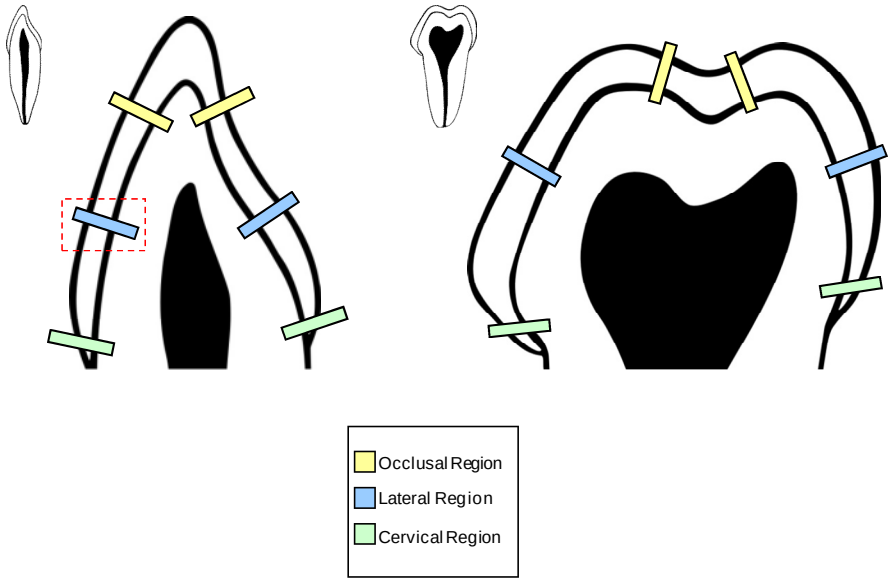


Figure 5.1: Diagrams of longitudinal sections of a deciduous canine and molar showing how each section was divided into occlusal, lateral and cervical regions. This was done on both the labial/buccal and lingual aspects of each tooth so that each tooth was represented by six separate regions of enamel. Twenty deciduous tooth crowns were divided up in this way. The labial lateral region of the canine section outlined by the rectangle of broken red lines represents the region shown below in **Figures 5.2** and **5.4**.

The smaller teeth, such as the incisors and canines required fewer photographs to span the entire prism length than the molars did as their enamel was thinner. The smallest possible number of photographs was always taken per region to ensure that any possible errors which may have occurred during the montage assembly stage were kept to a minimum. It was often possible to record the thinner layers of enamel such as those found in the cervical areas with just one photograph while the areas of thicker cuspal enamel required more photographs and resulted in longer montages. Depending on the enamel region and the size of the tooth that was being recorded, one to a maximum of eight photographs was required to produce a photomontage along the complete prism length.

A photograph of a graticule slide scored with 10µm increments over 1mm (1000µm) was also always taken on the same film at the same magnification and conditions as the ground sections in order to enable the subsequent calibration of the montage by using the graticule as a standard universal measurement.

Once the prints had been developed the montages were assembled. Each overlapping print was precisely matched to its neighbour. At least four prominent features on one print were identified and then located on the overlapping print; the clearer of the two prints was selected to be the upper most print. It was possible to alternate quickly between the two prints to ensure that an accurate match had been achieved and that there was an uninterrupted continuation of the enamel prisms, great care was taken to achieve an exact match between each photograph. Once achieved, the two prints were stuck together using double-sided adhesive tape, the join at the back of the two prints was further secured with 'Sellotape' to ensure that the prints stayed permanently in the correct position. A print of the graticule scale, taken with each run of prints was attached to each set of six photomontages for each ground section.

5.2.2 Production of Photomontages - Sample Group Two

The montages for sample group two were constructed at a lower magnification from a series of overlapping photographic prints taken with an Olympus OM-2N camera loaded with Kodak Gold 200 film attached to a Carl Zeiss Janamed 2 light microscope with a planachromat Pol 2.5/0.05 ∞/-A objective lens; from a 5 x 7 inch print of the negative the resultant field width was 4060µm.

Photographs were taken of the complete enamel crown in order to identify the exact position of the neonatal line. The photographs were taken along the entire crown from the tip of the cervical enamel on one aspect of the crown, up through the lateral enamel and occlusal enamel and down the other aspect of the crown to finish at the cervical tip on the opposite side. As it is the neonatal line and its position that is being examined in this part of the study, when the line was encountered through the camera lens, an attempt was made to 'fit' the entire line onto one photographic print by manipulating the view finder frame of the camera or the microscope slide, so that if errors were to occur during the construction of the montage, they would not be through the path of the neonatal line. Polarised light was utilised to help illustrate the neonatal line at its clearest and brightest. This process was repeated for ten ground sections of each tooth type. Ten photomontages were produced of the complete enamel crown for

each tooth type, resulting in the construction of 50 photomontages; which were constructed as described above.

The smaller teeth, such as the incisors and canines required fewer photographs than the molars did as their crowns were smaller. The smallest possible number of photographs was always taken per crown to ensure that any possible errors which may have occurred during the montage assembly stage were kept to a minimum. Depending on the size of the enamel crown that was being recorded, three to a maximum of eight photographs were required to produce a photomontage of the complete enamel crown.

A photograph of a graticule slide scored with 10µm increments over 1mm (1000µm) was also always taken on the same film at the same magnification and conditions as the ground sections in order to enable the subsequent calibration of the montage by using the graticule as a standard universal measurement.

5.3 Data Collection

Once constructed, the photomontages were then examined in greater detail. The examination of each set of montages for a given ground section was completed in one session, so as to decrease the possibility of observer error within each ground section.

5.3.1 Recording and Calculation of Cumulative Cross-Striation Counts to Produce Linear Regression Plots – Sample Group One

In order to ascertain the total number of cross-striations present along an enamel prism a ‘cumulative prism cross-striation count’ was made. Following the methods described previously for permanent occlusal enamel (Dean 2004; Dean 2007; Dean et al. 2001) a straight scale line was drawn on each photomontage running in the general prism direction within each region, care was taken to position the scale line along the majority of the prism path from its

beginning at the EDJ to its termination at the tooth surface. The length of the scale line was divided up into 100µm measurements (of enamel prism length) between the EDJ and the enamel surface using the photographic print of the graticule slide scored with 10µm increments (see **Figure 5.2**). If the enamel stopped short of a 100µm measurement at the surface, a 50µm measurement was recorded, this way as much of the prism length as possible was examined.

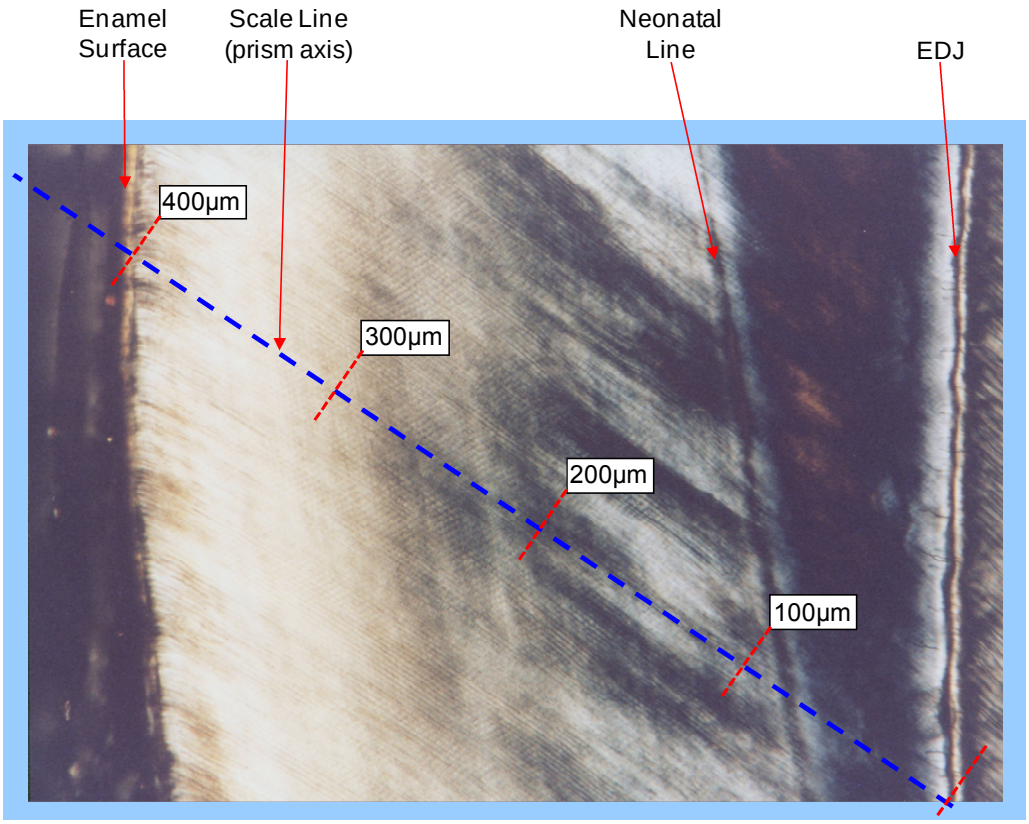


Figure 5.2: The lateral region of the labial aspect of a deciduous canine (see **Figure 5.1** above). The enamel surface, scale line along the prism axis (broken blue line), neonatal line and enamel-dentine junction (EDJ) are indicated. The distance along the prism axis from the EDJ to the enamel surface is measured and divided up to create either 100µm measurements or a final 50µm measurement (short broken oblique red lines crossing the long broken blue line), at each of these locations a cross-striation count was made and cumulated.

The prisms that ran alongside and/or close by this straight scale line path were then examined in detail. Counts of daily cross-striations were made commencing from the EDJ for each consecutive 100µm increment of the scale line; this was continued and cumulated along the length of the prism until the surface of the enamel was reached.

Each cross-striation was carefully marked onto the montage with a fine-tipped permanent Staedtler Lumocolor pen, therefore allowing this count to be verified

and double checked. Where cross-striations were not always visible along the length of the same prism, the adjacent prism was used and the counts were transcribed onto this prism. When the use of a neighbouring prism was unavoidable, great care was taken not to add or subtract increments and so introduce errors into the final count. The number of counts of the daily increments at the point where the prism passed each of the 100µm measurements on the scale line was cumulated from the EDJ to the enamel surface.

Prisms do not always travel in a straight path, especially close to the EDJ, they tend to weave or undulate, probably in three-dimensions, along a general prism path or direction (Dean 2004; Dean 2007; Dean et al. 2001). Where prisms weave around considerably there are more days between each of the 100µm measurements than in places where the prism course is almost straight and parallel to the scale line. Thus the time taken to form any given prism length was recorded in a way that took account of the sinuous nature of the prism path in two-dimensions (Dean 2004; Dean 2007; Dean et al. 2001).

Cumulative counts of daily increments were made along prisms from the EDJ along the scale line to the enamel surface. This was done on both the labial/buccal and lingual aspects of the ground section and in the occlusal, lateral and cervical regions of enamel resulting in a total of six prism trajectories for each of the four ground sections of each tooth type.

A number of linear regression plots were then made to explore the general trends in prism length in micrometers against formation time in days. Since convention holds that time is usually plotted on the x-axis to study growth curves, this first series of regression plots was generated with the enamel formation time (number of cross-striations i.e. days) as the independent variable (x-axis) against the cumulative enamel prism length (in micrometers) as the dependant variable (y-axis). The linear regression plots were generated from this data for both aspects (labial/buccal and lingual) and each enamel region (cervical, lateral and occlusal) for each tooth type.

The slopes of the plots, that describe the rates of enamel formation, were then analysed using *Statview (Abacus System™)*, in order to identify any statistical differences between each aspect of a tooth type as well as between the three enamel regions of each tooth type. Having determined which data could justifiably be combined and which showed significant differences, a new series of linear regression equations were then generated that combined more of the data for each aspect and/or region of each tooth type but this time using time in days as the dependant (y-axis) in order to generate linear regression formulae to predict enamel formation from the measurements along the enamel prism. The way that these regression formulae were then used is described below in **Section 5.3.2**.

5.3.2 Recording and Calculation of Crown Formation Times – Sample Group Two

In this sample of ground sections (sample group two), it was not possible to make continuous counts of cross-striations in the enamel. However, a clear neonatal line and other striae of Retzius or accentuated markings meant that total crown formation times could be calculated using the linear regression formulae derived from the previous sample (sample group one).

In order to determine the total time taken for crown formation in the different tooth types, first the formation of prenatal enamel (i.e. that under or beneath the neonatal line) was calculated, followed by the formation of postnatal enamel (i.e. that above or beyond the neonatal line). Each of these values were then added together to give the total enamel crown formation time.

Representation of the Neonatal Line

Once the photomontages had been constructed, a sheet of clear acetate was placed over the occlusal surface of the crown covering the neonatal line and secured firmly in place with ‘Sellotape’. The position of the neonatal line was then traced onto the acetate using a fine-tipped permanent Staedtler Lumocolor pen. Care was taken to make constant reference to the original ground section whilst this was being done in order to ensure that the neonatal line was

accurately identified on the photomontage and so correctly traced onto the acetate.

Crown and Neonatal Line Reconstruction

Although the least worn teeth were selected for this part of the study, some level of incisal and occlusal wear was unavoidable as few deciduous teeth are ever lost or shed naturally in an unworn state. Deciduous enamel is less hard than permanent enamel and so tends to wear very quickly, this is particularly noticeable in the anterior teeth as they are first to erupt and are therefore in the mouth for the longest period of time. In order to reconstruct the missing portions of the crown, the method described by Saunders et al. (2007:737) using *Adobe Photoshop*™ for cusp reconstruction was adapted for use on deciduous crowns and neonatal lines. This method of crown reconstruction involved first manually drawing two straight lines extending from the original unworn labial/buccal and lingual enamel surfaces onto the clear acetate until they intersected above the cusp. However, instead of using *Adobe Photoshop*™ as suggested by Saunders et al. (2007), a third line was drawn onto the acetate from the middle of the highest point of the dentine horn through the point of the intersection of the first two lines. The 'simulated' crown outline was then drawn in free-hand along the first two lines ensuring that the highest point of the simulated incisal/occlusal edge was on the third line drawn from the dentine horn to the intersection of the first two lines and so was directly inferior to the point of intersection. In order to validate this method of crown reconstruction, it was first carried out on several montages of unworn teeth and then subsequently applied to each of the photomontages from sample group two in turn; including the unworn crowns in order to practice and refine the technique. The line of the EDJ was also used as a guide to help reconstruct the 'simulated' crown surface. In addition, slides of unworn teeth of the same tooth type were continually referred to in order to help reconstruct the original position of the enamel surface as accurately as possible.

This method of crown reconstruction for permanent teeth, described by Saunders et al. (2007) adapted well to deciduous crown reconstruction. As this method of reconstruction worked well on the montages of the unworn teeth and

the ‘simulated’ crown cusps corresponded closely with true unworn crown cusps, it was felt that the results obtained using this reconstruction method were as accurate and reliable as could be achieved. This method was further extended when required, to the reconstruction of the neonatal line using the contours of the enamel surface and the EDJ as a guide.

Due to the varying degree of incisal and occlusal wear, all of the crowns and 18 out of 50 of the neonatal lines were reconstructed to some extent (see **Table 5.2**); although this reconstruction method appeared reliable, wherever possible the neonatal line and subsequent striae were always examined and measured from the original montage rather than the reconstruction. Although the occlusal surfaces were reconstructed in every case, this does not influence the subsequent measurements taken in any way, only in the cases of the reconstructed neonatal lines over the cusp tips is there the possibility of the introduction of any measurement error.

Table 5.2: This table shows the number of incisal and occlusal crown surfaces and neonatal lines that were reconstructed. The reconstruction of the neonatal line may influence the final crown formation calculations, as on occasions these measurements were recorded from the reconstruction rather than the original neonatal line.

Tooth Type (10 of each type)	Crown Reconstruction	Neonatal Line Reconstruction
A	10	7
B	10	1
C	10	4
D	10	6
E	10	0
Total	**50**	**18**

The selected 32 unworn and 18 reconstructed neonatal lines where then used in the following analysis.

In order to determine the total crown formation times of deciduous enamel, the labial/buccal aspect was selected to be examined in greater detail. This was due to the fact that labial/buccal enamel is thicker than lingual enamel and so contains the greatest number of increments of growth from initiation at the EDJ until the end of enamel formation at the labial/buccal cervix. Therefore in a forensic context the buccal aspect is of more use when trying to establish an estimated age of an individual, as it potentially offers a longer time-line than the

lingual enamel does. The buccal aspect was also shown to be more statistically constant during the analysis of the regression formulae (see **Section 6.1.3** and **6.1.5**).

5.3.2.a Crown Formation before Birth – Beneath the Neonatal line

Mitutoyo Absolute Digimatic digital callipers, accurate to ±0.02mm were used to take all of the measurements from the photomontages. In order to determine the maximum number of days taken to form the prenatal enamel, the buccal aspect was examined in each of the photomontages. A measurement was taken from the EDJ along the length of a prism to the first appearance of the neonatal line and recorded (Distance *A*). This measurement in millimetres was taken along the length of a clear enamel prism as close to the occlusal tip as possible, just before the enamel became too gnarled and decussated to follow easily over the tip of the dentine horn and wherever possible avoiding any reconstruction of the neonatal line. This is also where the prenatal enamel is at its thickest and therefore has been forming for longest. This measurement was repeated three times and the mean was recorded on the acetate.

The graticule scale photograph was used to convert the mean millimetre measurements obtained using the callipers (Distance *A*) to micrometers (μm) as follows:

In order to convert the measurements taken with the callipers in millimetres (*A* mm) into micrometers (μm) the photographic print of the graticule scale was used.

For example:
1000μm on the graticule scale photograph was 42.99mm

Therefore

$$\frac{1000}{42.99} = 23.26\mu m$$

So
1mm on the photomontage = 23.26 micrometers

Therefore
(Distance *A*) x 23.26 = prenatal enamel prism length in micrometers

The converted mean distance measured from the EDJ to the neonatal line was then entered into the linear regression formulae that were derived from the previous cumulative counts of daily incremental cross-striations made at regular 100µm intervals in ground sections from sample group one (see previous **Section 5.3.1**). Prenatal enamel formation times were calculated in this way for each of the buccal aspects of the ten photomontages for each tooth type, resulting in 50 individual prenatal enamel formation times.

5.3.2.b Crown Formation after Birth – Beyond the Neonatal line

In order to determine the maximum number of days taken to form the postnatal enamel, the buccal aspect was again examined in each of the photomontages. Following a method described previously for permanent teeth (Dean 1998; Risnes 1986) the point of the termination where the neonatal line intersected the EDJ was used as the initial base point for estimating the postnatal enamel formation time. From this point the enamel prism closest to the termination of the neonatal line was followed outward from the EDJ along the prism path until a pronounced stria was encountered (see **Figure 5.3**). The distance along the prism from the end of the neonatal line to the first stria was measured using digital callipers and was recorded (Distance *B*). The location of this stria was traced onto the clear acetate and followed back until it reached the EDJ and then the process was repeated. The enamel prism closest to the termination of the previous stria, was followed outwards from the EDJ along the prism path to the next pronounced stria and again this distance was measured and recorded (Distance *C*), this second stria was again traced back to its termination at the EDJ. This process was repeated until no more striae were encountered and the surface of the enamel was reached, these measurements were again recorded (Distance *D, E* etc.). Finally the surface of the enamel was traced on to the acetate terminating at the cervical point of the crown. Three measurements were taken for each distance and then the mean was recorded on the acetate.

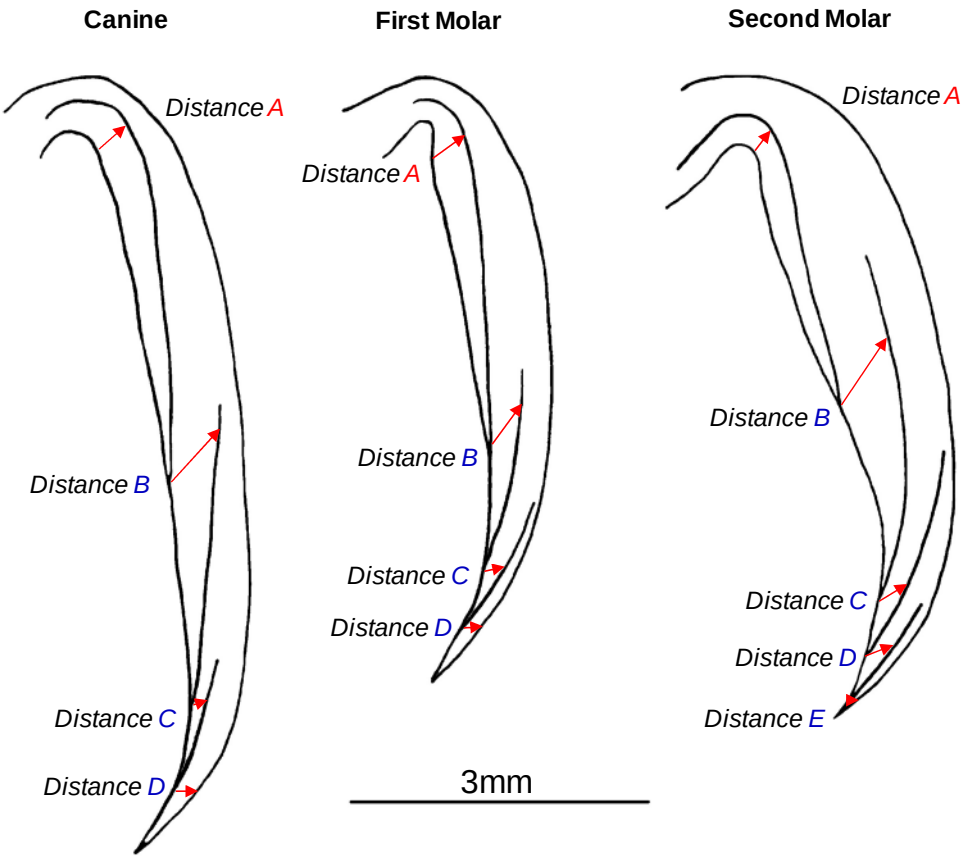


Figure 5.3: Diagrams made using the drawing tube, to show how the crown formation times, before and after birth were measured in the buccal aspect of the ten photomontages for each tooth type. Measurements were made from the EDJ along a prism path to the neonatal line or first accentuated stria, this neonatal line or stria was then traced back to the EDJ, where the process was repeated until the cervical point was reached. These measurements were made for several distances in each photomontage and then the total time taken for crown formation was calculated from these distances.

Each of the mean measurements obtained beyond the neonatal line to the cervix was then converted to micrometers as described above and then entered into the linear regression formulae. Total postnatal enamel formation times were calculated for each of the buccal aspects of the ten photomontages for each tooth type. The total postnatal enamel formation time was calculated using the following equation:

(*B*) + (*C*) + (*D*) + (*E* etc.) = *Total Postnatal Enamel Formation Time*

5.3.2.c Calculation of the Total Crown Formation Time

In order to calculate the total crown formation time the resultant number of days for prenatal enamel formation from **Section 5.3.2.a** were added to the resultant days from **Section 5.3.2.b**, as shown below.

Prenatal Enamel Formation Time + *Total Postnatal Enamel Formation Time* =
Total Crown Formation Time

The total crown formation times were calculated for each of the ten ground sections for each tooth type.

5.3.3 Measuring Enamel Formation Rates at Greater Resolution across the Neonatal Line – Sample Group One

So that the enamel formation rate across the neonatal line could be examined in greater detail, mean daily cross-striation measurements were made at regular intervals through the enamel thickness of each aspect, region and for each tooth type. Using the original 120 photomontages described above in **Section 5.3.1** each of the 100µm measurements was extended in height (occlusally and cervically) on the photomontage with a line parallel to the EDJ, crossing the scale line at each 100µm distance from the EDJ. If the enamel stopped short of a 100µm measurement at the enamel surface, a 50µm zone was recorded, this way as much of the enamel thickness as possible was examined. The entire enamel thickness was divided up into 100µm zones in this way either with or without a final 50µm zone at the surface (see **Figure 5.4**).

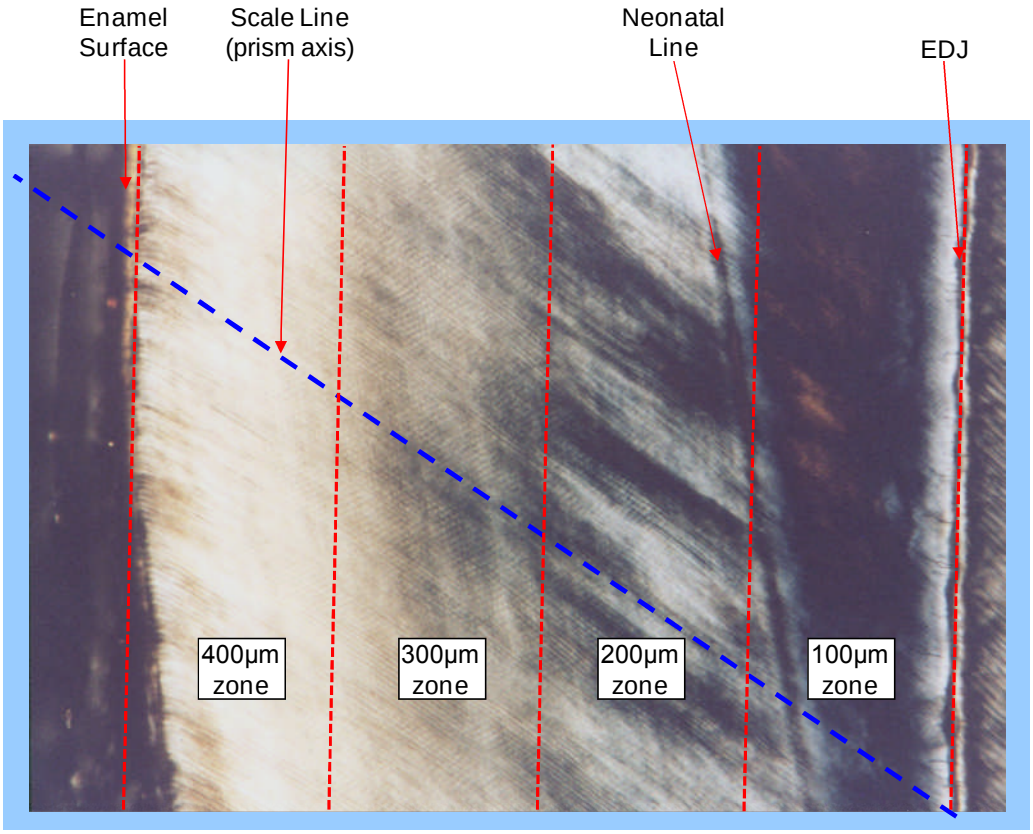


Figure 5.4: The lateral region of the labial aspect of a deciduous canine (see **Figure 5.1** above). The enamel surface, scale line along the prism axis (broken blue line), neonatal line and enamel-dentine junction (EDJ) are indicated. The distance along the prism axis from the EDJ to the enamel surface is measured, to create 100µm zones (with or without a final 50µm surface zone) parallel to the EDJ (broken red lines).

Within each of the 100µm zones, the distance between a consecutive series of six cross-striations (which represents five days continuous enamel growth) was measured and the mean was then calculated. Increment location marks, made with a fine-tipped permanent Staedtler Lumocolor pen, were made below the actual increments as it was easier to identify these marks than it was to locate the exact centre of the increment when measuring (see **Figure 5.5**). This procedure was repeated ten times, evenly spaced across each zone; throughout the entire thickness of the enamel (five sets of the six incremental counts were made either side of the original cumulative count scale line). All measurements made on the montages were rounded up to the nearest 0.1µm.

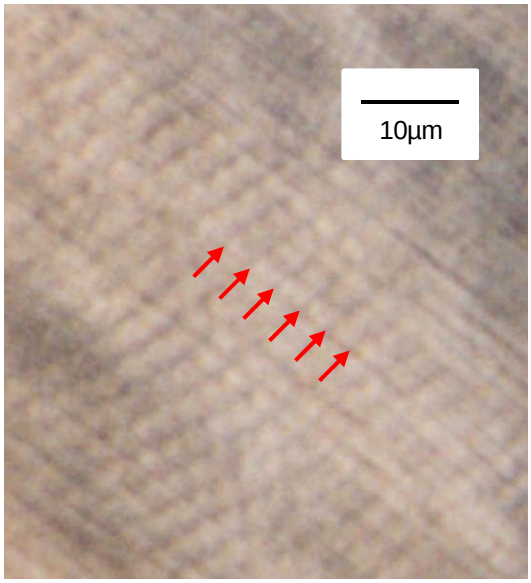


Figure 5.5: A magnified section of **Figure 5.4** illustrating an example of six cross-striations (five continuous days of enamel growth).

Each count of six was always taken from a single photographic print rather than from an area where two photographs had been joined together. This was done in order to prevent any possible error from occurring that may have resulted from minute discrepancies during montage construction. The scale for each set of six photomontages was calculated using the photographic print of the graticule and then the mean daily incremental growth was calculated as follows:

In order to determine the mean daily incremental growth of each of these groups of six cross-striations, the measurements taken with the callipers in millimetres were converted into micrometers (µm) using the photographic print of the graticule scale.

For example:
300µm on the graticule scale photograph is 131.20mm

Therefore

$$\frac{300}{131.20} = 2.29\mu m$$

So
1mm on the photomontage = 2.29µm

This measurement in micrometers was then used to calculate the mean daily incremental rate of each of the calliper readings.

For example:

$$\frac{\text{Distance between 6 incremental markings}}{\text{Number of Days (5)}} = \text{Amount Of Growth Per Day}$$

Therefore
Six incremental markings (5 days growth) = 6.07mm

$$\frac{6.07}{5} \times 2.29 = 2.78\mu m$$

So
The average daily incremental growth rate of enamel in this example is 2.78 micrometers per day across 5 daily increments.

The data for the ten mean values in each 100µm or final 50µm zone were presented as box plots for each aspect and region and for each of the four ground sections from each tooth type. Starting from the EDJ and progressing to the enamel surface, this continuous series of average daily cross-striation spacings were used to identify any gradients or sudden changes in the rate of enamel formation. In order to determine whether the presence of the neonatal line had any influence on the enamel formation rate, reference was made back to the ground sections. This reference to the ground sections to identify and clarify the exact position of the neonatal line on the photomontages was made after all of the series of counts had been obtained so as not to inadvertently influence any of the measurements. The distance between the EDJ and the neonatal line as it first crossed the scale line was then measured (see broken blue line in **Figure 5.4** above) and superimposed onto the box plots.

It became evident that the presence of some other pronounced striae, possibly produced at times of stress during the development of the enamel, also seemed to coincide with decreased rates of enamel growth and so the ground sections were further examined for the presence of ‘stress lines’. Each ground section was used directly in conjunction with its corresponding photomontage to locate the exact position of any ‘stress lines’ and then to superimpose these onto the box plots.

CHAPTER 6: Results

6.1 Cumulative Rates of Enamel Formation – Sample Groups One and Two

In order to ascertain the total number of cross-striations present along an enamel prism, 'cumulative cross-striation counts' were taken every 100µm along the prism length, from the enamel-dentine junction to the enamel surface on both aspects (labial/buccal and lingual) and in three regions (cervical, lateral, and occlusal). These tabulated results can be found in **Appendix One**.

For the initial analysis of these growth data, both within a tooth type and between tooth types, the convention of plotting time on the x-axis was followed. First, a scattergram (**Figure 6.1a**) with enamel formation time (number of daily cross-striations) as the independent variable was plotted against the cumulative prism lengths (in micrometers) as the dependant variable. In **Figure 6.1b** the same data were then split by tooth type. Next, a scattergram for each individual tooth type was generated for both the anterior (**Figure 6.2a**) and posterior (**Figure 6.2b**) teeth, which were also then split by tooth type (**Figure 6.3**). In **Figure 6.4** scattergrams for each tooth type, split by their individual ground sections, were then generated in order to try to identify whether any spread of data was due in part to any of the individual sections.

For each of these plots a simple linear regression line was fitted and in **Figure 6.1a** upper and lower 95% confidence limits are also shown. In no case did fitting a polynomial regression curve improve the value of R^2[10] and so simple linear regression equations were judged appropriate. In all cases the coefficient of determination (R^2) was greater than 0.90 and the P-value >0.001. The values of R^2 and the regression equation for each plot appear with each plot along with an explanatory key when the data were split. Since the correlation coefficient (r^2) is always greater than the coefficient of determination (R^2) that is shown here for each plot and since R^2 is so high for all plots, r^2 was not calculated.

[10] The closer the R^2 value is to 1, the greater the ability of the model is to predict a trend.

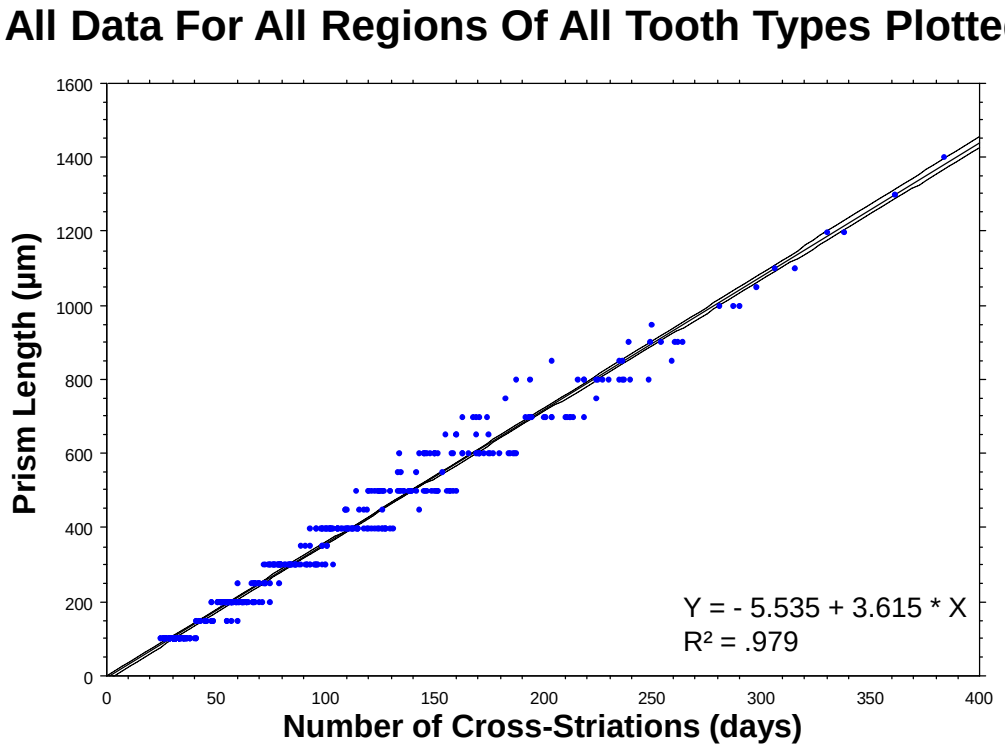


Figure 6.1a: This bivariate scattergram with the regression line and 95% confidence limits for the mean, shows the data obtained for all aspects and regions of the crown combined for all five tooth types.

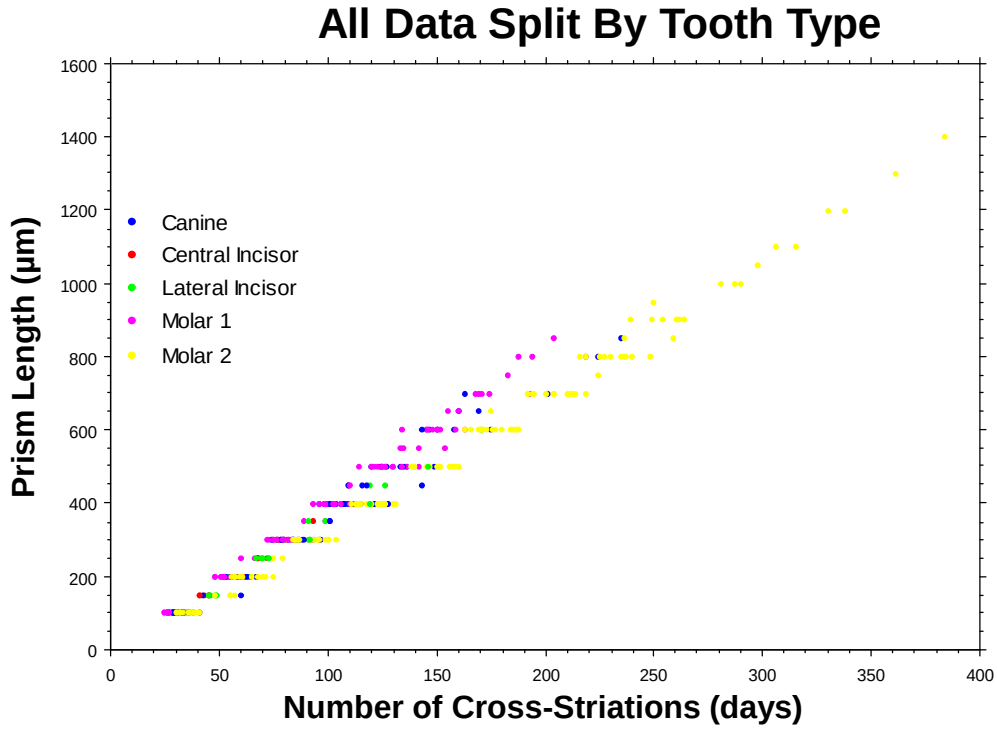


Figure 6.1b: This bivariate scattergram shows the data obtained for all aspects and regions of the crown split by tooth type.

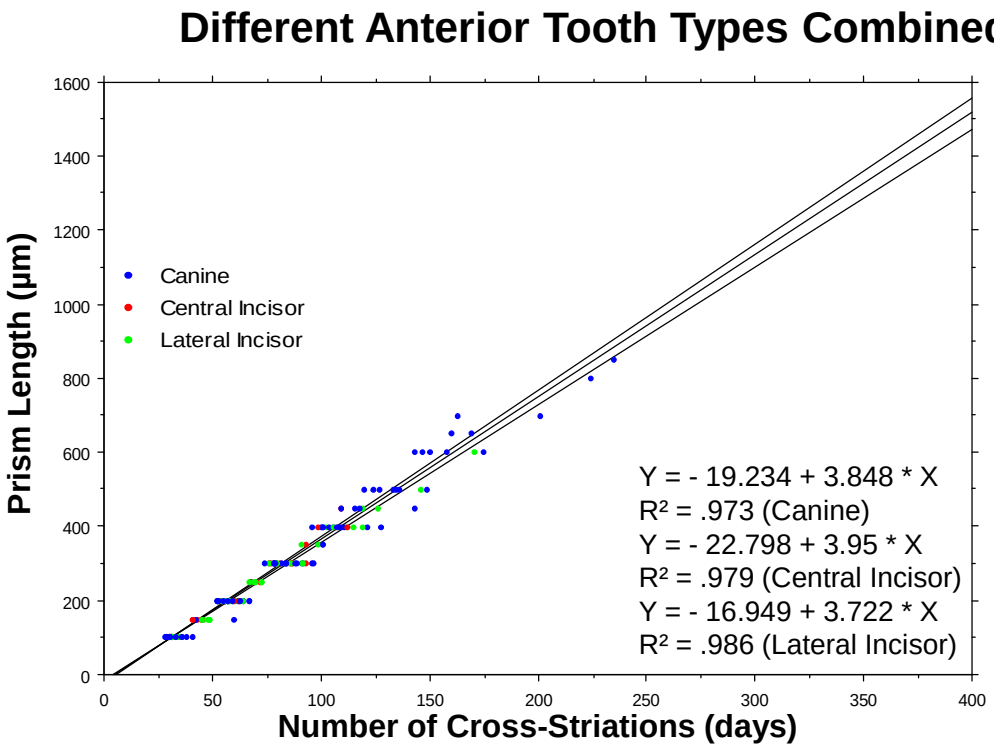


Figure 6.2a: This bivariate scattergram with the regression line for each tooth type shows the data obtained for individual anterior teeth, with combined aspects and regions.

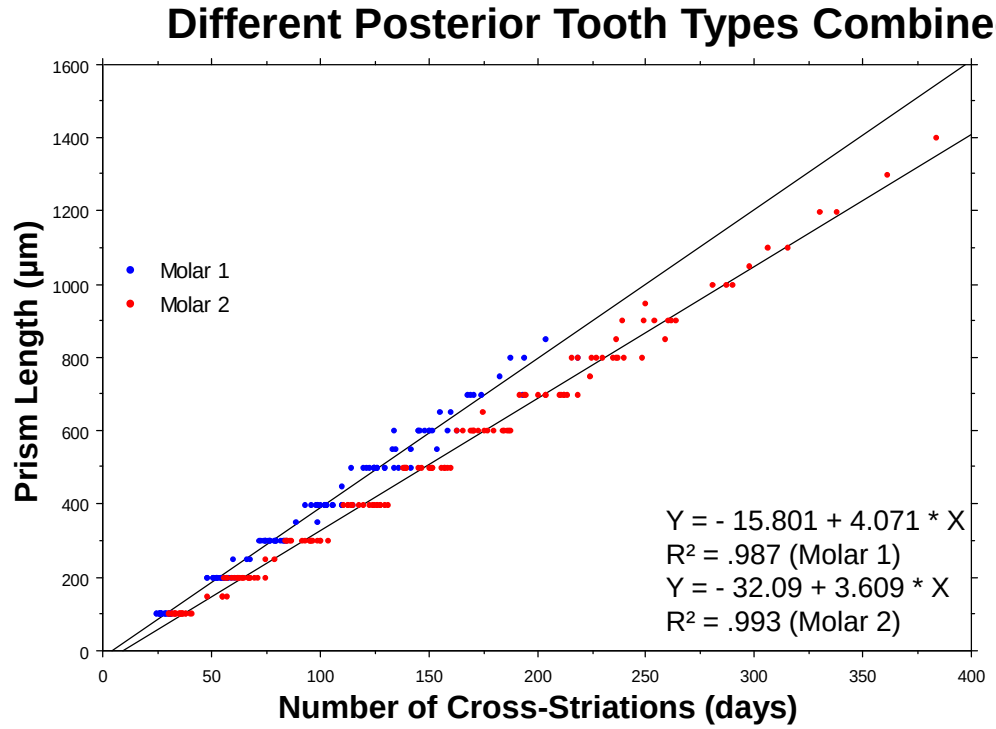


Figure 6.2b: This bivariate scattergram with the regression line for each tooth type shows the data obtained for individual posterior teeth, with combined aspects and regions.

All Aspects And Regions Of Each Tooth Type Plotted Together

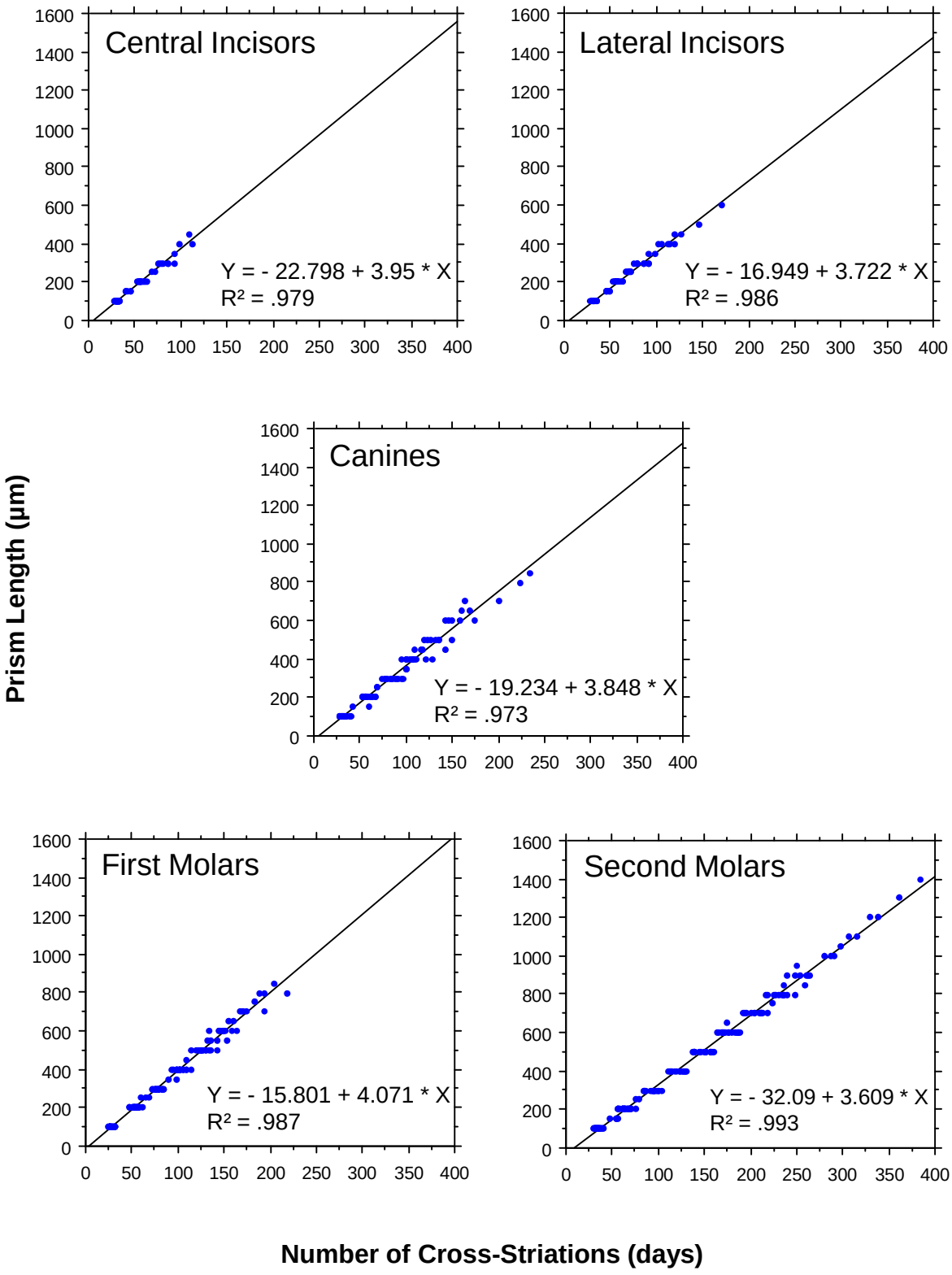


Figure 6.3: These bivariate scattergrams with the regression line show the data obtained for each tooth type, with combined aspects and regions.

Individual Ground Sections Within Each Tooth Type

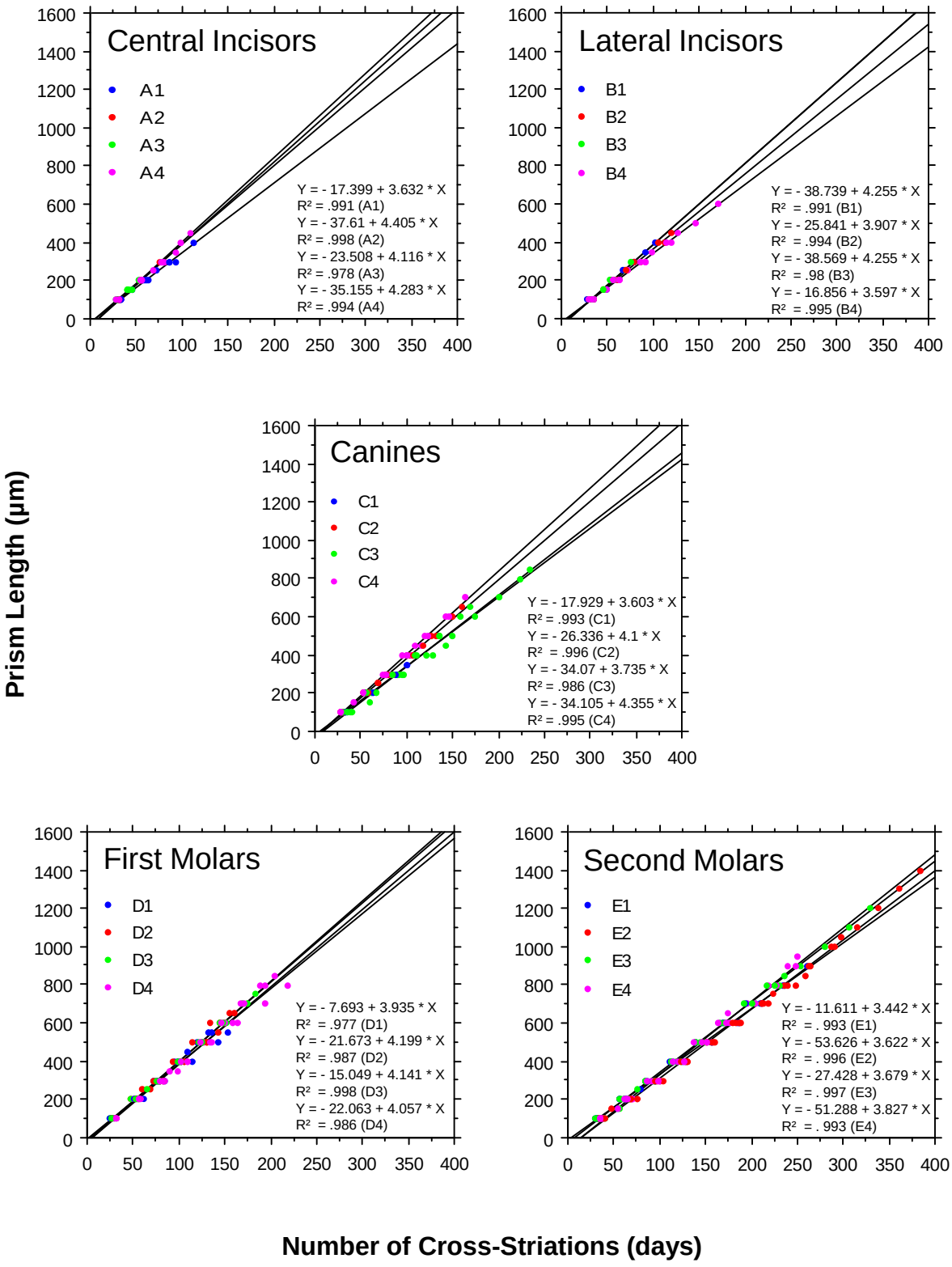


Figure 6.4: These bivariate scattergrams with the regression line for each ground section show the data obtained for each tooth type, with combined aspects and regions.

6.1.1 Summary Discussion

The resultant plots (see **Figure 6.1**), clearly show that there is a tight positive correlation between the prism length and the number of cross-striations counted. In each sample, there is an increase in variation as the enamel formation time increases across all tooth types, which is typical of all growth data. The main point illustrated in **Figure 6.2** is that the molar teeth appear to form two distinct groups either side of the regression line, with the first molar appearing to develop faster than the second molar. **Figure 6.3** shows enamel formation rates within the individual tooth types. Again there is a tight positive correlation between the prism length and the number of enamel cross-striations. To explore the possible effects of any one tooth on the sample, plots of each tooth type were split by section. **Figure 6.4** clearly shows that individual variation plays a large part in the dispersal of the data around the regression line. The best examples of this are in the central incisors where slide A1 is clearly separated from the other teeth and in the canines where C3 is responsible for causing this dispersal. C3 is interesting as this individual was from the Spitalfields collection and is of an archaeological nature, when compared to the other canine teeth the rate of enamel formation is slower in this tooth. Although this enamel is thicker than that of the 'modern' teeth, this osteology collection is essentially of pathological origin and it is therefore not unreasonable to suggest that the dental development of this individual may also have been affected by malnutrition and illness similar to the adult skeletons that were excavated with this juvenile. This sad state of health may have resulted in the slower rate of dental development that is shown in **Figure 6.4**.

6.1.2 Statistical Analysis to Identify the Presence of Any Differences in the Rate of Enamel Formation between Crown Aspects

In order to help visualize any differences or similarities between the two aspects of each tooth type, plots of each, split by aspect are shown in **Figure 6.5**. Independent t-tests were performed to determine whether the labial/buccal and lingual aspect of each tooth differed significantly in the number of cross-striations present (see **Table 6.1**). Since three tests were performed for each tooth (one for each region), the p-value for the level of significance 0.05 was corrected to $p<0.0167$ (Bonferroni correction) in order to avoid increased type 1 error frequency[11].

Table 6.1: This table shows the independent sample t-tests calculated to identify any significant differences between the two dental aspects in each region for each tooth type.

Central Incisor

Aspects	Region	Mean (µm)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference (µm)		Significance (2-tailed)
					Lower	Upper	
Labial Lingual (N = 4)	Cervical	1.250	1.258	0.629	-0.752	3.252	0.141
Labial Lingual (N = 7)	Lateral	1.857	2.410	0.911	-0.372	4.086	0.088
Labial Lingual (N = 8)	Occlusal	0.750	1.832	0.648	-0.782	2.282	0.285

[11] The p-value is a number between 0 and 1 that reflects the strength of the data that are being used to evaluate the null hypothesis. If the p-value is small, then there is strong evidence against the null hypothesis, while a large p–value indicates weak evidence against the null hypothesis.

Lateral Incisor

Aspects	Region	Mean (µm)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference (µm)		Significance (2-tailed)
					Lower	Upper	
Labial Lingual (N = 5)	Cervical	0.400	2.702	1.208	-2.955	3.755	0.757
Labial Lingual (N = 7)	Lateral	1.000	2.309	0.873	-1.136	3.136	0.296
Labial Lingual (N = 9)	Occlusal	1.222	3.866	1.289	-1.749	4.194	0.371

Canine

Aspects	Region	Mean (µm)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference (µm)		Significance (2-tailed)
					Lower	Upper	
Labial Lingual (N = 6)	Cervical	-0.333	2.160	0.882	-2.600	1.934	0.721
Labial Lingual (N = 15)	Lateral	1.333	5.367	1.386	-1.639	4.306	0.352
Labial Lingual (N = 14)	Occlusal	-3.000	7.514	2.008	-7.339	1.339	0.159

First Molar

Aspects	Region	Mean (µm)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference (µm)		Significance (2-tailed)
					Lower	Upper	
Buccal Lingual (N = 11)	Cervical	4.000	4.899	1.477	0.709	7.291	0.022
Buccal Lingual (N = 28)	Lateral	6.929	7.328	1.385	4.087	9.770	0.000
Buccal Lingual (N = 20)	Occlusal	5.300	4.835	1.081	3.037	7.563	0.000

Second Molar

Aspects	Region	Mean (µm)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference (µm)		Significance (2-tailed)
					Lower	Upper	
Buccal Lingual (N = 4)	Cervical	-1.000	5.354	2.677	-9.520	7.520	0.734
Buccal Lingual (N = 30)	Lateral	-1.433	4.523	0.826	-3.122	0.256	0.093
Buccal Lingual (N = 27)	Occlusal	-0.519	6.818	1.312	-3.216	2.179	0.696

N= number of paired aspects

Any value less than 0.0167 indicates a highly significant difference in the number of cross-striations between the two aspects. Only the first molar shows any statistical indication that there may be a difference between the buccal and lingual aspects (see **Figure 6.5**). This difference was apparent in the lateral and the occlusal regions. The other four tooth types did not express any significant differences between the labial/buccal and lingual aspects.

Unfortunately as the sample size was too small (i.e. less than 5) in the cervical regions of the central incisors and the second molars, an accurate test of significance could not be determined, however as the lateral and occlusal areas of both of these teeth were not significantly different it seems reasonable to suggest that the whole tooth behaves in the same manner and that there was no significant difference between the labial/buccal and lingual aspects in these teeth.

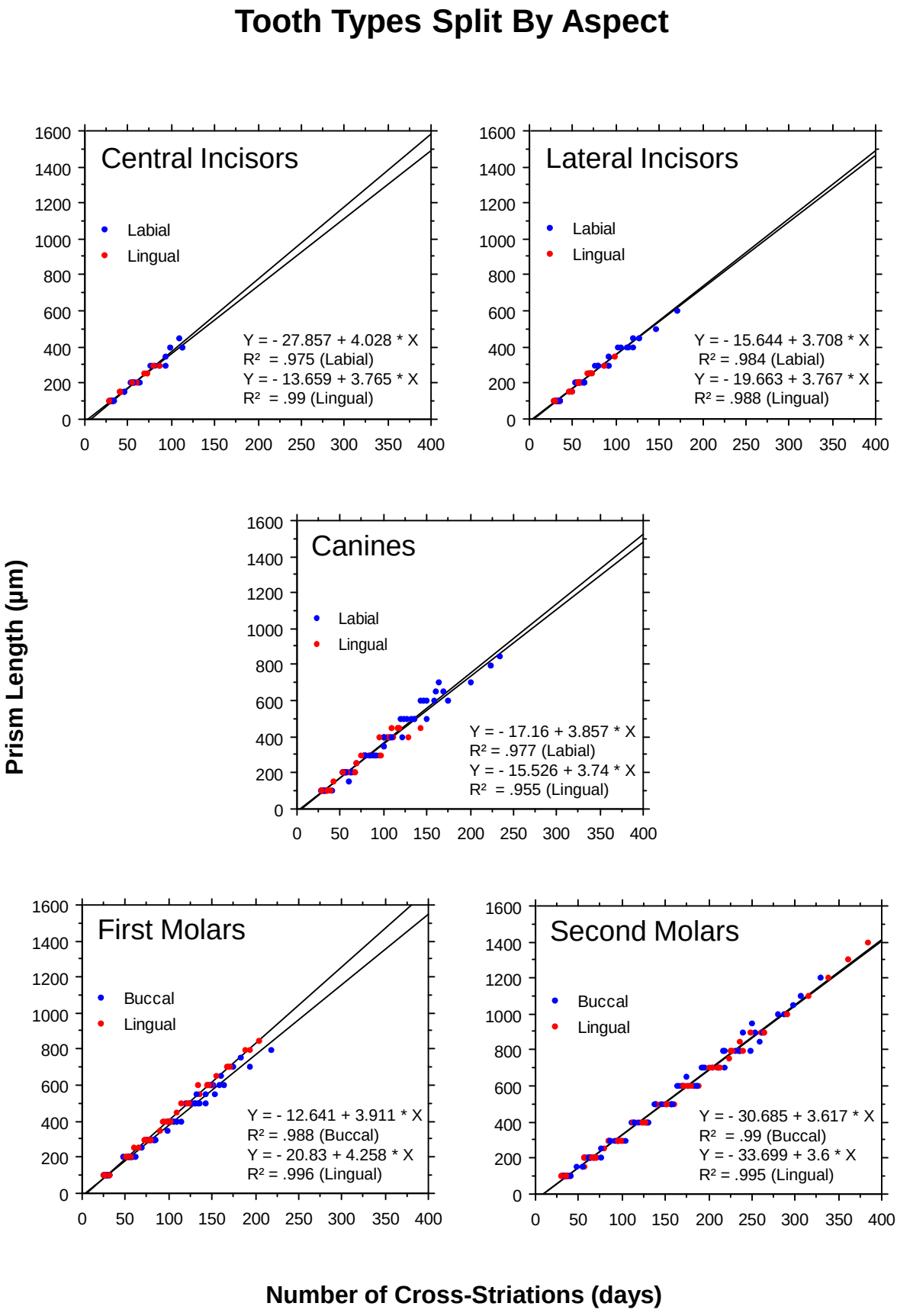


Figure 6.5: These bivariate scattergrams with the regression line show the data obtained for each tooth type split by aspect.

6.1.3 Summary Discussion

The resultant plots (**Figure 6.5**), show the individual tooth types separated by their crown aspects. The independent t-tests confirmed that only the first molar showed any statistically significant differences with the buccal enamel appearing to develop at a slower rate than the lingual enamel. This difference appears in both the lateral and the occlusal regions. The other four tooth types showed no significant differences between the labial/buccal and lingual aspects.

Based on the above results, both aspects of the crown of each tooth type were then treated as being identical and these data were merged together. This merging of the data both increases the sample size and creates a statistically sound data set for the subsequent analyses. In the case of the first molar, the data from the lateral and occlusal regions on the lingual aspect were eliminated from the subsequent analyses. Data from the buccal aspect were retained as the enamel on this aspect is thicker than that on the lingual aspect and it contains the greatest record of time from initiation at the dentine horn until the end of enamel formation at the cervix. Therefore, in a forensic context, the buccal aspect is of more use when trying to establish an estimated age at death, as it potentially offers a longer time-line than the lingual enamel.

6.1.4 Statistical Analysis to Identify the Presence of Any Differences in the Rate of Enamel Formation between the Crown Regions

In order to help visualise any differences or similarities between the three regions of each tooth type, plots of each split by region are shown in **Figure 6.6** and regions spilt by tooth type are shown in **Figure 6.7**. Three paired t-tests were performed to determine whether there was any significant difference between the three different regions (cervical, lateral and occlusal) on each dental aspect for each tooth type (see **Table 6.2**). The p-value for the level of significance 0.05 was again corrected to $p<0.0167$ (Bonferroni correction) in order to avoid increased type 1 error frequency.

Tooth Types Split By Region

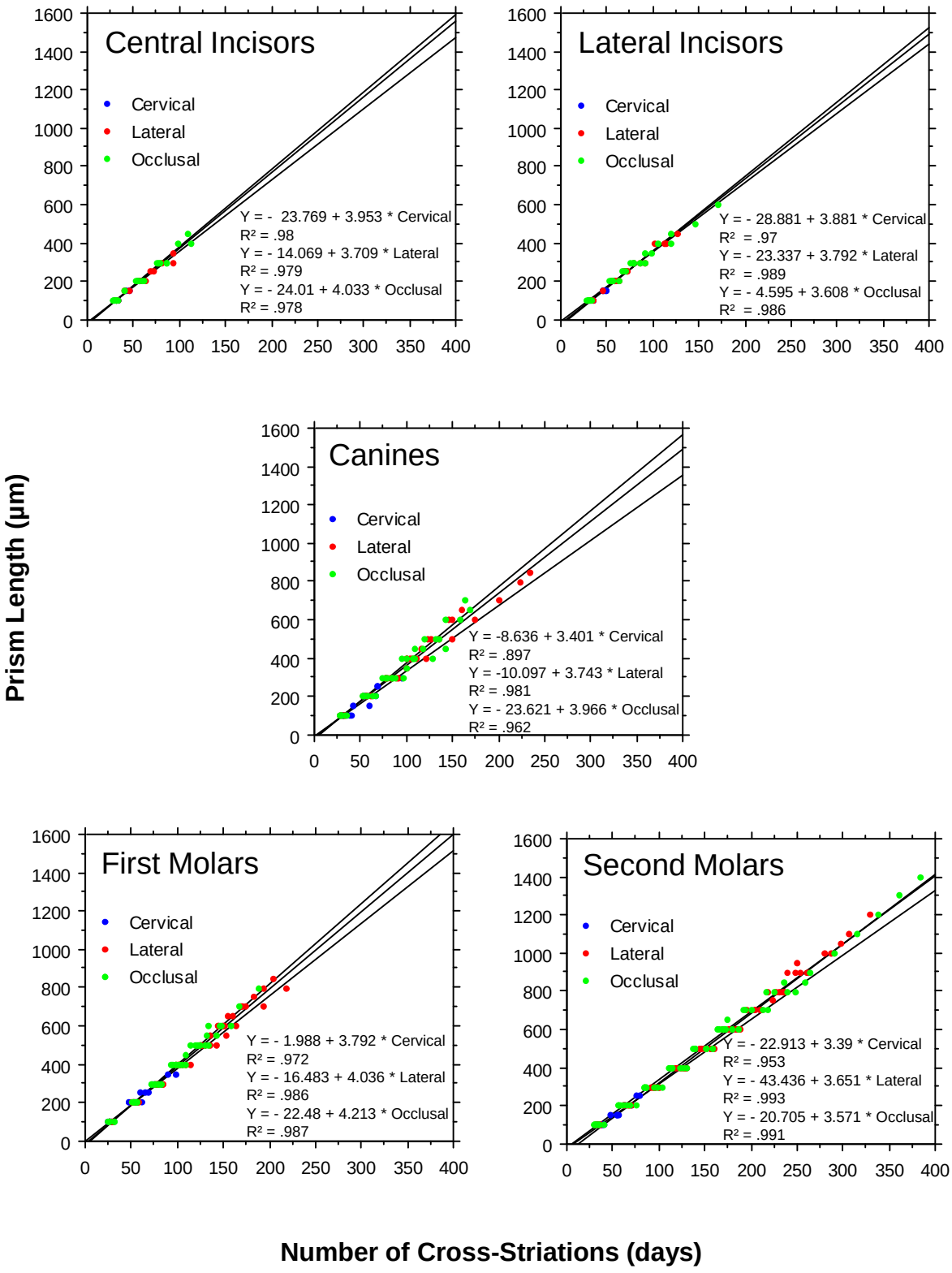
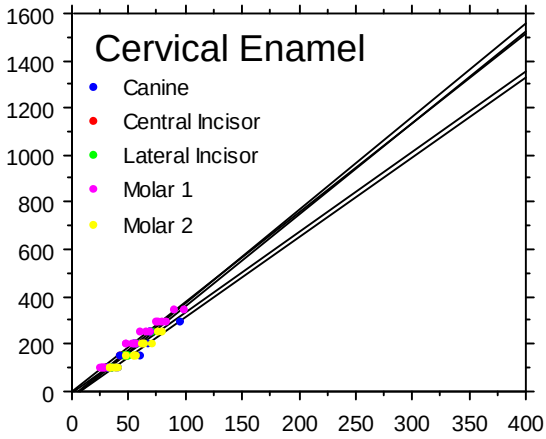
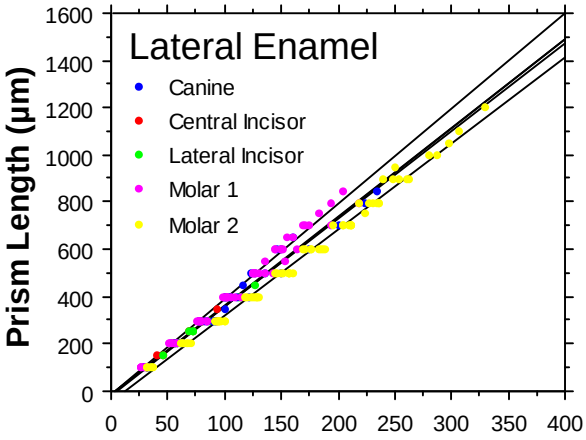


Figure 6.6: These bivariate scattergrams with the regression line show the data obtained for each tooth type split by region.

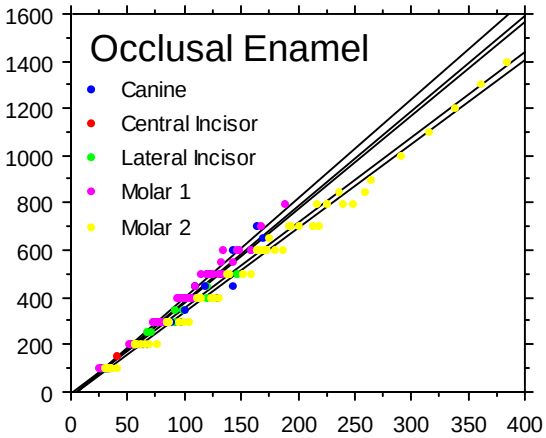
Regions Split By Tooth Types



Y = - 8.636 + 3.401 * X
R^2 = .897 (Canine)
Y = - 23.769 + 3.953 * X
R^2 = .98 (Central Incisor)
Y = - 28.881 + 3.881 * X
R^2 = .97 (Lateral Incisor)
Y = - 1.988 + 3.792 * X
R^2 = .972 (Molar 1)
Y = - 22.913 + 3.39 * X
R^2 = .953 (Molar 2)



Y= - 10.097 + 3.743 * X
R^2 = .981 (Canine)
Y = - 14.069 + 3.709 * X
R^2 = .979 (Central Incisor)
Y= - 23.337 + 3.792 * X
R^2 = .989 (Lateral Incisor)
Y= - 16.483 + 4.036 * X
R^2 = .986 (Molar 1)
Y= - 43.436 + 3.651 * X
R^2 = .993 (Molar 2)



Y = - 23.621 + 3.966 * X
R^2 = .962 (Canine)
Y = - 24.01 + 4.033 * X
R^2 = .978 (Central Incisor)
Y = - 4.595 + 3.608 * X
R^2 = .986 (Lateral Incisor)
Y = - 22.48 + 4.213 * X
R^2 = .987 (Molar 1)
Y = - 20.705 + 3.571 * X
R^2 = .991 (Molar 2)

Number of Cross-Striations (days)

Figure 6.7: These bivariate scattergrams with the regression line show the data obtained for each region split by tooth type.

Table 6.2: This table shows the paired sample t-tests calculated to identify any significant differences between the three regions on the same dental aspect for each tooth type.

Central Incisor

Regions	Aspect	Mean (µm)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference (µm)		Significance (2-tailed)
					Lower	Upper	
Cervical Lateral (N = 4)	Labial	0.250	1.258	0.629	-1.752	2.252	0.718
Cervical Lateral (N = 6)	Lingual	0.000	1.414	0.577	-1.484	1.484	1.000
Cervical Occlusal (N = 4)	Labial	0.750	1.500	0.750	-1.637	3.137	0.391
Cervical Occlusal (N = 6)	Lingual	0.000	2.098	0.856	-2.201	2.201	1.000
Lateral Occlusal (N = 9)	Labial	1.667	2.179	0.726	-0.009	3.342	0.051
Lateral Occlusal (N = 7)	Lingual	0.429	1.718	0.649	-1.161	2.018	0.534

Lateral Incisor

Regions	Aspect	Mean (µm)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference (µm)		Significance (2-tailed)
					Lower	Upper	
Cervical Lateral (N = 6)	Labial	-1.667	4.227	1.726	-6.103	2.769	0.378
Cervical Lateral (N = 5)	Lingual	0.800	1.304	0.583	-0.819	2.419	0.242
Cervical Occlusal (N = 6)	Labial	0.333	4.676	1.909	-4.574	5.241	0.868
Cervical Occlusal (N = 4)	Lingual	2.250	4.113	2.056	-4.295	8.795	0.354
Lateral Occlusal (N = 13)	Labial	2.000	3.162	0.877	0.089	3.911	0.042
Lateral Occlusal (N = 8)	Lingual	2.250	2.252	0.796	0.367	4.133	0.026

Canine

Regions	Aspect	Mean (µm)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference (µm)		Significance (2-tailed)
					Lower	Upper	
Cervical Lateral (N = 6)	Labial	2.000	3.521	1.438	-1.695	5.695	0.223
Cervical Lateral (N = 7)	Lingual	4.286	4.716	1.782	-0.076	8.647	0.053
Cervical Occlusal (N = 6)	Labial	3.333	4.033	1.647	-0.899	7.566	0.099
Cervical Occlusal (N = 7)	Lingual	1.000	2.309	0.873	-1.136	3.136	0.296
Lateral Occlusal (N = 21)	Labial	2.905	5.726	1.250	0.298	5.511	0.031
Lateral Occlusal (N = 15)	Lingual	-1.000	8.150	2.104	-5.514	3.514	0.642

First Molar

Regions	Aspect	Mean (µm)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference (µm)		Significance (2-tailed)
					Lower	Upper	
Cervical Lateral (N = 10)	Buccal	-0.800	3.259	1.031	-3.131	1.531	0.458
Cervical Lateral (N = 10)	Lingual	-0.200	3.425	1.083	-2.650	2.250	0.858
Cervical Occlusal (N = 10)	Buccal	-0.700	4.762	1.506	-4.107	2.707	0.653
Cervical Occlusal (N = 10)	Lingual	-0.200	3.824	1.209	-2.935	2.535	0.872
Lateral Occlusal (N = 23)	Buccal	3.348	7.444	1.552	0.129	6.567	0.042
Lateral Occlusal (N = 23)	Lingual	2.913	4.542	0.947	0.949	4.877	0.006

Second Molar

Regions	Aspect	Mean (µm)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference (µm)		Significance (2-tailed)
					Lower	Upper	
Cervical Lateral (N = 5)	Buccal	0.600	4.722	2.112	-5.263	6.463	0.790
Cervical Lateral (N = 7)	Lingual	1.286	4.071	1.539	-2.479	5.051	0.435
Cervical Occlusal (N = 5)	Buccal	0.800	4.604	2.059	-4.917	6.517	0.717
Cervical Occlusal (N = 7)	Lingual	3.857	4.947	1.870	-0.718	8.433	0.085
Lateral Occlusal (N = 29)	Buccal	4.862	8.564	1.590	1.605	8.120	0.005
Lateral Occlusal (N = 24)	Lingual	4.375	4.312	0.880	2.554	6.196	0.000

Any value less than 0.0167 indicates a highly significant difference in the number of cross-striations between the different regions on the same dental aspect, only the first and second molars showed any significant differences between the different regions of the same dental aspect. This difference was apparent in the lateral and occlusal regions of both molars. In the first molar there was a significant difference on the lingual aspect between the lateral and occlusal regions but not between these regions on the buccal aspect. In the second molar there was no significant difference on the buccal or lingual aspect between the cervical and lateral regions or between the cervical and occlusal regions. There was, however, a significant difference between the lateral and occlusal regions on both aspects.

Unfortunately, the sample size was too small (i.e. less than 5) in the cervical/lateral and cervical/occlusal comparisons of the labial aspect of the central incisor and in the cervical/occlusal comparison of the lingual aspect of the lateral incisor, to obtain an accurate measure of statistical significance. However, as the other regions on the same aspect of both of these teeth showed no significance it seems reasonable to suggest that the whole tooth

grew enamel in the same manner with no discernible differences between regions on the same aspect.

6.1.5 Summary Discussion

Based on the above results, all three regions on the labial and lingual aspects of the central and lateral incisors and of the canines were treated as being identical. The data for each region were therefore combined for each tooth type in subsequent analyses. In the case of the first molar the combined data for all regions of the buccal aspect and the cervical region on the lingual aspect were used in subsequent analyses. For the second molar the statistical tests revealed that the *inner* rates of enamel formation were not significantly different from each other between regions of the crown (see also the results of the subsequent **Section 6.1.7** below). However, when the longer trajectories of the occlusal and lateral enamel were compared, the diverging rates of outer enamel formation caused the occlusal and lateral regions to become significantly different (see **Figure 6.7**) As a result of this it was decided to exclude the occlusal data completely and to combine the lateral and cervical data to generate a regression formula to predict enamel formation times in the second molar. A condition of doing this was that enamel prism lengths longer than those in the lateral enamel could not be used in subsequent predictions of enamel formation times in the occlusal region. However, in practice, no lengths beyond the inner enamel were ever measured in second molars (or in any other tooth type) in this study.

6.1.6 Generating Regression Formulae to Predict Enamel Formation Time

In order to generate regression equations to predict the number of days of enamel formation from a given prism length formed from the EDJ, time (days of formation) was now plotted as the dependant variable on the y-axis. This was done with all data for all regions and aspects combined as defined above. In addition, regression formulae for the upper and lower 95% confidence limits were generated as well. The plots for each tooth type are shown in **Figure 6.8**

and the resultant regression formulae are set out in **Table 6.3**. The regression formulae for the combined data from each tooth type were used to construct a comparative table of enamel formation times for given lengths measured from the EDJ along a prism path (**Table 6.3**). This table gives predictions for enamel formation times in days for each 10µm measurement of enamel prism length between 50µm and 100µm and then for each subsequent 50µm measurement of prism length for each deciduous tooth type. The ranges of enamel formation times predicted using the formulae for the upper and lower 95% confidence limits are also presented.

6.1.7 Summary Discussion

The table of predicted enamel formation times shows that the number of days taken to form a small length of enamel prism near to the EDJ in each tooth type is very similar (**Table 6.3**). Indeed, even the upper and lower 95% confidence limits are only a few days different to each of the mean predictions. However, as prism length increases, bigger differences begin to emerge between each tooth type. At, for example, at a prism length of 800µm in deciduous canines and in first and second molars, the predictions for the number of days to form this length of prism are 210, 206 and 231 days respectively. These observations suggest first, that more accurate predictions are likely for shorter rather than longer prism lengths from the EDJ, but also that the enamel growth trajectories (the slopes of the plots) for different tooth types are indeed slightly different to one another. This becomes more important were one to use these regression formulae to predict the time taken to form longer lengths of enamel prism in different deciduous tooth types.

Regression Plots Used To Predict Enamel Formation Times

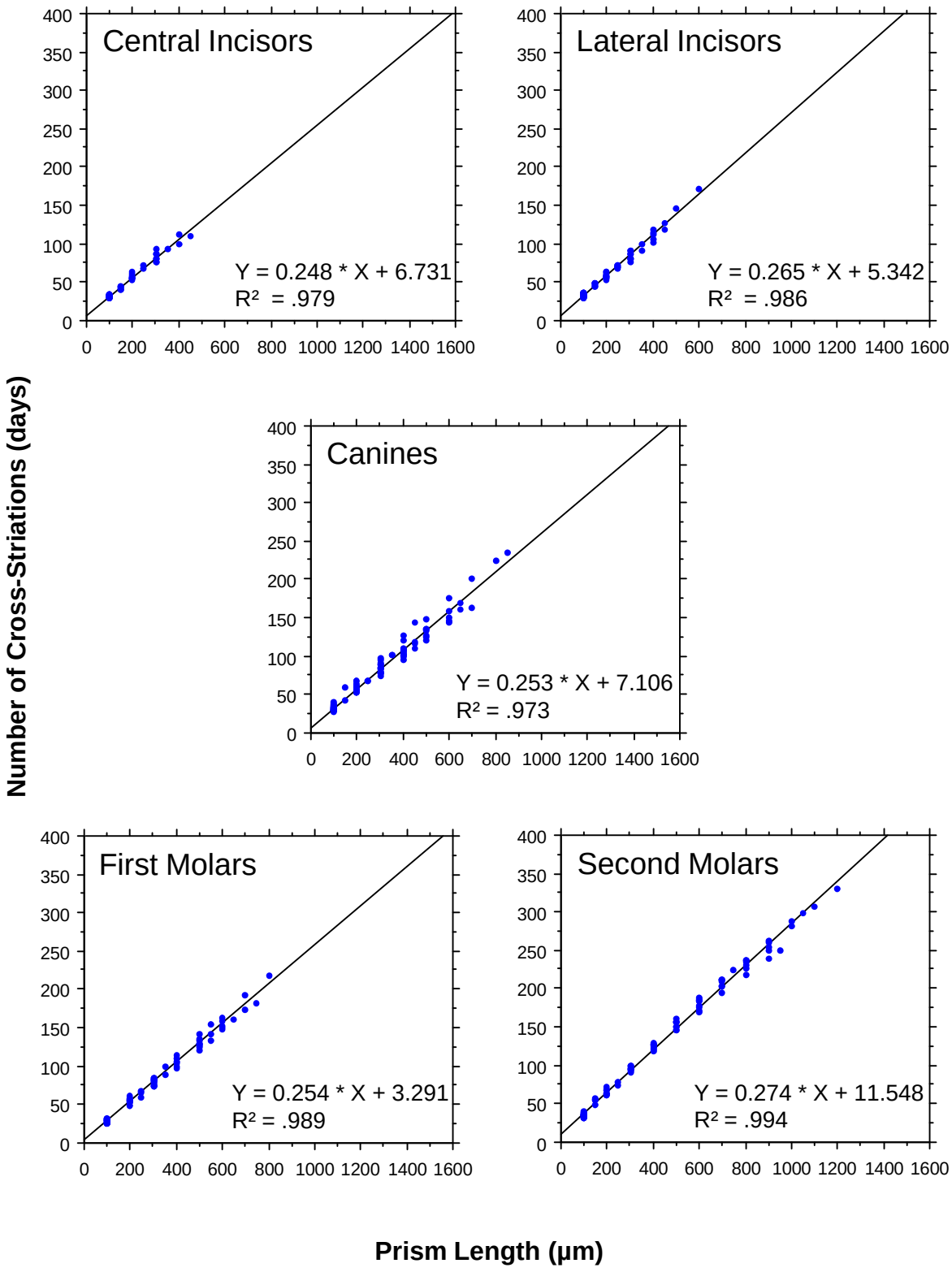


Figure 6.8: These bivariate scattergrams with the regression lines show the data used as the basis for predicting enamel formation time (days) from prism length measurements (µm) from the EDJ.

Table 6.3: This table presents values for the formation time in days of each 10µm measurement of enamel thickness starting from 50µm to 100µm and then for each subsequent 50µm measurement for each deciduous tooth type. The data were generated using the regression formulae developed in **Section 6.1.6**. They give the mean time of enamel formation in days (Y) as well as the ranges predicted by the upper and lower 95% confidence limits.

Central Incisors

Enamel Thickness (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Range (days)
50	19	17	22	2.6
60	22	19	24	2.7
70	24	21	27	2.8
80	27	24	29	2.9
90	29	26	32	3.0
100	32	28	35	3.1
150	44	40	47	3.6
200	56	52	60	4.1
250	69	64	73	4.6
300	81	76	86	5.1
350	94	88	99	5.6
400	106	100	112	6.1
450	118	112	125	6.6

Mean
Y = 0.248 x Enamel Thickness (µm) + 6.731
Lower 95% Confidence Limit
Y = 0.238 x Enamel Thickness (µm) + 4.678
Upper 95% Confidence Limit
Y = 0.258 x Enamel Thickness (µm) + 8.784

Lateral Incisors

Enamel Thickness (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Range (days)
50	19	16	21	2.2
60	21	19	23	2.2
70	24	22	26	2.3
80	27	24	29	2.4
90	29	27	32	2.4
100	32	29	34	2.5
150	45	42	48	2.9
200	58	55	62	3.2
250	72	68	75	3.6
300	85	81	89	3.9
350	98	94	102	4.3
400	111	107	116	4.6
450	125	120	130	5.0
500	138	133	143	5.3
550	151	145	157	5.7
600	164	158	170	6.0

Mean
Y = 0.265 x Enamel Thickness (µm) + 5.342
Lower 95% Confidence Limit
Y = 0.258 x Enamel Thickness (µm) + 3.535
Upper 95% Confidence Limit
Y = 0.272 x Enamel Thickness (µm) + 7.148

Canines

Enamel Thickness (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Range (days)
50	20	16	23	3.4
60	22	19	26	3.5
70	25	21	28	3.6
80	27	24	31	3.7
90	30	26	34	3.8
100	32	29	36	3.9
150	45	41	49	4.3
200	58	53	62	4.8
250	70	65	75	5.2
300	83	77	88	5.7
350	96	90	101	6.1
400	108	102	114	6.6
450	121	114	128	7.0
500	134	126	141	7.5
550	146	138	154	7.9
600	159	151	167	8.4
650	172	163	180	8.8
700	184	175	193	9.3
750	197	187	206	9.7
800	210	199	219	10.2
850	222	212	232	10.6

Mean
Y = 0.253 x Enamel Thickness (µm) + 7.106
Lower 95% Confidence Limit
Y = 0.244 x Enamel Thickness (µm) + 4.123
Upper 95% Confidence Limit
Y = 0.261 x Enamel Thickness (µm) + 10.089

First Molars

Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Range (days)
50	16	13	19	2.6
60	19	16	21	2.7
70	21	18	24	2.8
80	24	21	26	2.8
90	26	23	29	2.9
100	29	26	32	2.9
150	41	38	45	3.2
200	54	51	58	3.5
250	67	63	71	3.8
300	79	75	84	4.1
350	92	88	97	4.4
400	105	100	110	4.7
450	118	113	123	5.0
500	130	125	136	5.3
550	143	137	149	5.6
600	156	150	162	5.9
650	168	162	175	6.2
700	181	175	188	6.5
750	194	187	201	6.8
800	206	199	214	7.1

Mean
Y = 0.254 x Prism Length (µm) + 3.291
Lower 95% Confidence Limit
Y = 0.248 x Prism Length (µm) + 0.951
Upper 95% Confidence Limit
Y = 0.260 x Prism Length (µm) + 5.631

Second Molars

Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Range (days)
50	25	23	28	2.6
60	28	25	31	2.7
70	31	28	33	2.7
80	33	31	36	2.8
90	36	33	39	2.8
100	39	36	42	2.9
150	53	50	56	3.1
200	66	63	70	3.4
250	80	76	83	3.6
300	94	90	97	3.9
350	107	103	111	4.1
400	121	117	125	4.4
450	135	130	139	4.6
500	149	144	153	4.9
550	162	157	167	5.1
600	176	171	181	5.4
650	190	184	195	5.6
700	203	197	209	5.9
750	217	211	222	6.1
800	231	224	236	6.4
850	244	238	250	6.6
900	258	251	264	6.9
950	272	265	278	7.1
1000	286	278	292	7.4
1050	299	292	306	7.6
1100	313	305	320	7.9
1150	327	319	334	8.1
1200	340	332	348	8.4

Mean
Y = 0.274 x Prism Length (µm) + 11.548
Lower 95% Confidence Limit
Y = 0.269 x Prism Length (µm) + 9.194
Upper 95% Confidence Limit
Y = 0.278 x Prism Length (µm) + 13.902

6.1.8 Use of Linear Regression Formulae to Estimate Crown Formation Times - Sample Group Two

The measurements (described in **Section 5.3.2**) taken from the photomontages from the EDJ to points on prism paths intersecting with the neonatal line and other subsequently formed striae in the crown were entered into the regression equations described in the previous section. Each formula was used to calculate the time taken to form prenatal and postnatal enamel for each of the ten ground sections of each tooth type in sample group two. These results appear in **Appendix Two** and are also summarised below in **Table 6.4** together with the upper and lower 95% confidence limits and the percentage of crown formed before birth and after birth.

6.1.9 Summary Discussion

Table 6.4 shows that the range of total crown formation time estimates is greatest for the canines, with a possible range of 56 days and least in the lateral incisors, with a range of only 22 days. This predicted range of enamel formation times is more pronounced in the postnatal enamel, which is to be expected since prenatal enamel is relatively consistent in its slower inner enamel formation rates. Postnatal enamel also exhibits a greater number of pronounced (or accentuated) striae and goes on forming for a longer time. Although these striae are an essential part of the process of reconstructing crown formation times they are not always clear and are often difficult to trace in deciduous enamel. Their presence may also contribute to a decreased rate of enamel formation (see **Section 6.2.1** below) which may affect the accuracy of the predictions calculated from the regression formulae which are based on many teeth. Another issue to be considered is the occurrence of cross-striation ‘doubling’ in sample group one. This would affect the final predictions and would result in the ‘over aging’ of individual total enamel formation times. Although, when present, regions of ‘doubling’ were recognised and avoided, this is more common in postnatal enamel than in prenatal enamel. Prenatal enamel appears to form at a more regular and consistent rate.

Table 6.4: This table shows the mean results of the combined regression formulae when applied to ten ground sections of each tooth type. The crown formation times are expressed in days, weeks and months, as well as indicating the error margin in days (rounded to the nearest decimal place).

Central Incisor

			Confidence Limits				
	Mean (days)	% Crown Completion	95% Lower (days)	95% Upper (days)	Range (days)	Weeks (mean day/7)	Months (mean day/30.44)
Crown Formation Time Before Birth	144	59.99%	137	152	15	20.6	4.74
Crown Formation Time After Birth	96	40.01%	89	103	15	13.7	3.16
Total Crown Formation Time	240	100%	226	255	30	34.4	7.90

Lateral Incisors

			Confidence Limits				
	Mean (days)	% Crown Completion	95% Lower (days)	95% Upper (days)	Range (days)	Weeks (mean day/7)	Months (mean day/30.44)
Crown Formation Time Before Birth	136	54.81%	131	142	11	19.5	4.48
Crown Formation Time After Birth	113	45.19%	107	119	12	16.1	3.70
Total Crown Formation Time	249	100%	238	260	22	35.6	8.18

Canines

			Confidence Limits				
	Mean (days)	% Crown Completion	95% Lower (days)	95% Upper (days)	Range (days)	Weeks (mean day/7)	Months (mean day/30.44)
Crown Formation Time Before Birth	128	29.87%	121	135	14	18.4	4.22
Crown Formation Time After Birth	302	70.13%	280	322	42	43.1	9.91
Total Crown Formation Time	430	100%	401	458	56	61.4	14.13

First Molars

			Confidence Limits				
	Mean (days)	% Crown Completion	95% Lower (days)	95% Upper (days)	Range (days)	Weeks (mean day/7)	Months (mean day/30.44)
Crown Formation Time Before Birth	140	43.01%	135	146	11	20.0	4.61
Crown Formation Time After Birth	186	56.99%	175	197	21	26.5	6.10
Total Crown Formation Time	326	100%	310	342	33	46.6	10.71

Second Molars

			Confidence Limits				
	Mean (days)	% Crown Completion	95% Lower (days)	95% Upper (days)	Range (days)	Weeks (mean day/7)	Months (mean day/30.44)
Crown Formation Time Before Birth	118	23.23%	113	122	8	16.8	3.86
Crown Formation Time After Birth	389	76.77%	373	403	30	55.5	12.77
Total Crown Formation Time	506	100%	487	524	38	72.3	16.64

While it is less than the postnatal enamel formation range, the range of prenatal enamel formation times is interesting. The sample of deciduous teeth examined was a mixed random sample and there is no way of knowing if the distribution of gestation times was similar across all tooth types. A sample with more premature and late birth deliveries would result in a more varied prenatal enamel formation period. Another potential source of error relates to reconstructing the precise position of the neonatal line described in **Section 5.3.2**. The prenatal enamel formation range appears to be larger in the samples of teeth where more reconstructions were required. Among central incisors, which required seven neonatal lines to be reconstructed and first molars where six neonatal lines were reconstructed, prenatal enamel formation ranges were greater (11-15 days) than those in second molars where none of the neonatal lines required reconstruction and where the range of enamel formation time was smallest (eight days).

Each of these issues suggested that a more detailed study of enamel formation rates across the neonatal line and on into postnatal enamel formation might resolve some of the unanswered questions about the range of pre- and postnatal enamel formation times.

6.2 Enamel Formation Rates Across the Neonatal Line – Sample Group One

So that the enamel growth rate could be examined in greater detail and at a greater resolution, large numbers of mean daily cross-striation counts were taken throughout the enamel thickness of each region on each aspect and for each tooth type. To do this, the original 120 photomontages of sample group one were used. This time the enamel was divided into 100µm zones, each parallel with the EDJ (as described in **Section 5.3.3** above). The distance between a consecutive series of six cross-striations (representing five days enamel growth) was measured and the mean value calculated. The procedure was repeated ten times, evenly spaced throughout each of the 100µm zones throughout the entire thickness of the enamel. Identical data sets were collected within occlusal, lateral and cervical regions on both aspects of each of the photomontages. The results of this analysis appear in **Appendix Three** and are also presented here as box plots for each aspect and region for each of the four ground sections of each tooth type (see **Figures 6.9 – 6.18**).

The horizontal lines on each box indicate the 25^{th}, 50^{th} and 75^{th} centiles and the whiskers indicate the 10^{th} and 90^{th} centiles. Prenatal enamel is indicated as red box plots and postnatal enamel is indicated as blue. The position and distance of the neonatal line with respect to the EDJ is illustrated by a vertical broken red line.

The pattern of changing enamel formation rates for each aspect of each tooth are shown on separate graphs but the formation rates for the cervical, lateral and occlusal regions for the same tooth are shown on the same graph and are denoted by different coloured backgrounds in order to highlight the differences in their formation rates.

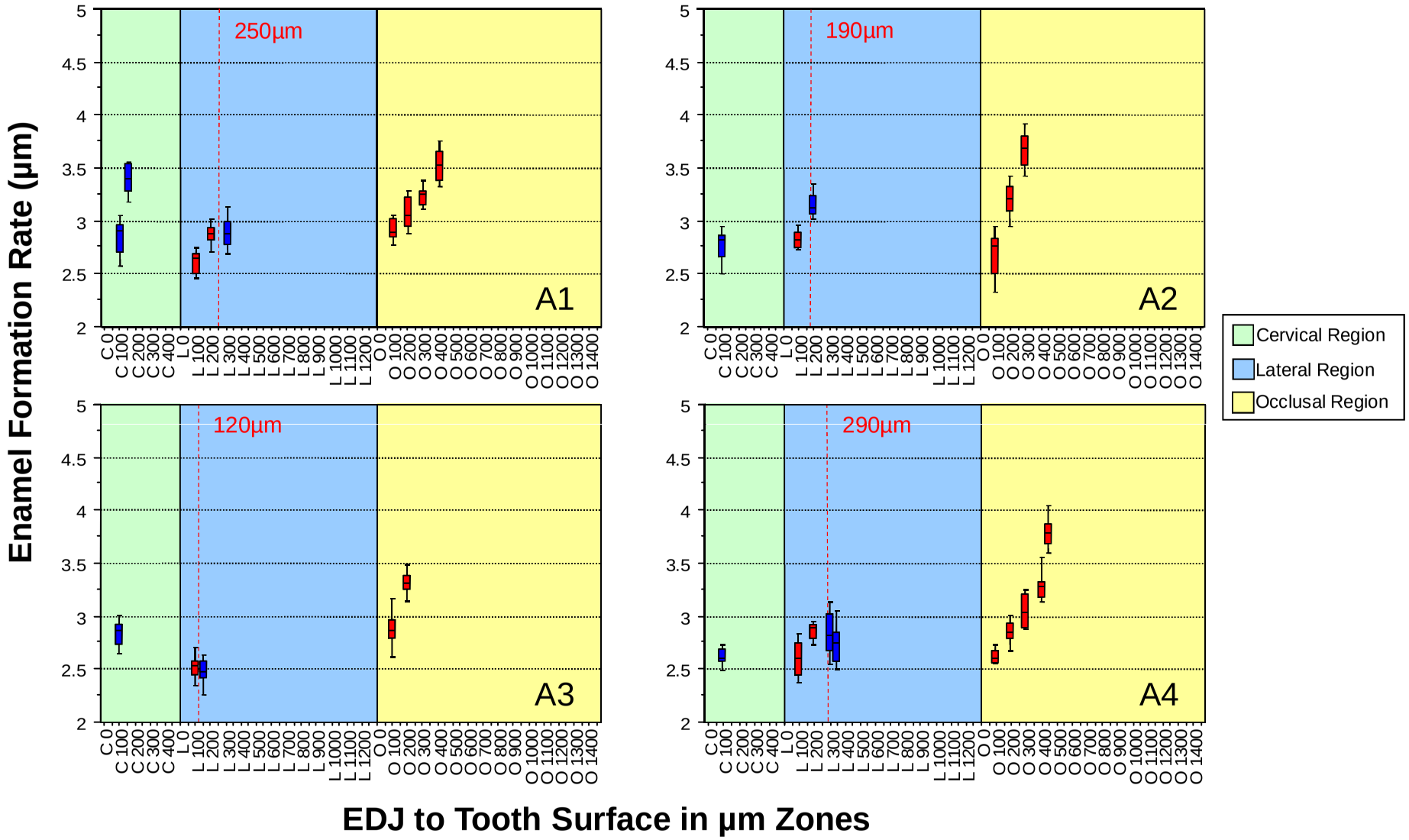


Figure 6.9: These box plots show the enamel formation rates for the labial aspect of four central incisors. Cervical, lateral and occlusal enamel regions are denoted by different shaded backgrounds. The vertical broken red line shows the position of the NNL with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

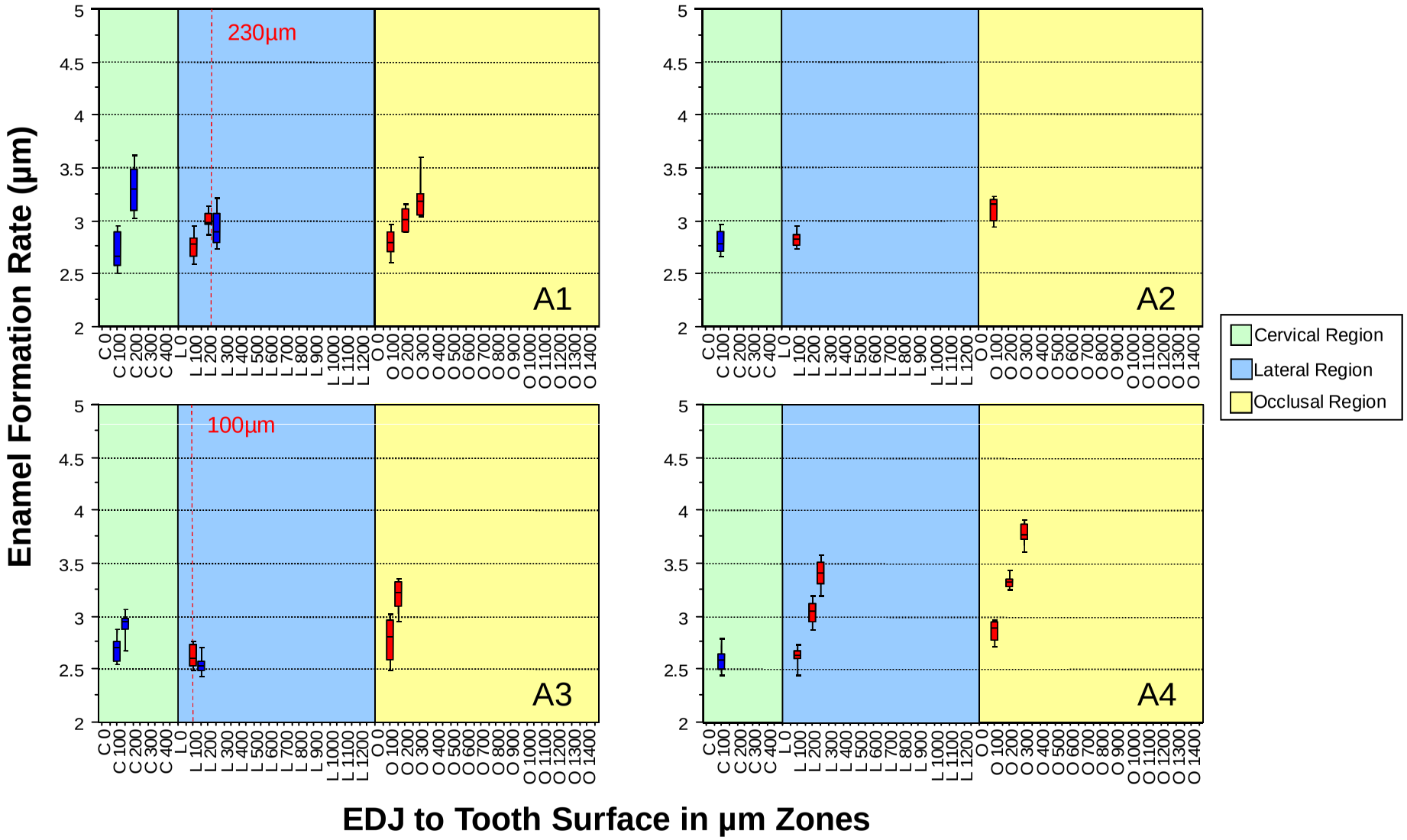


Figure 6.10: These box plots show the enamel formation rates for the lingual aspect of four central incisors. Cervical, lateral and occlusal enamel regions are denoted by different shaded backgrounds. The vertical broken red line shows the position of the NNL with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

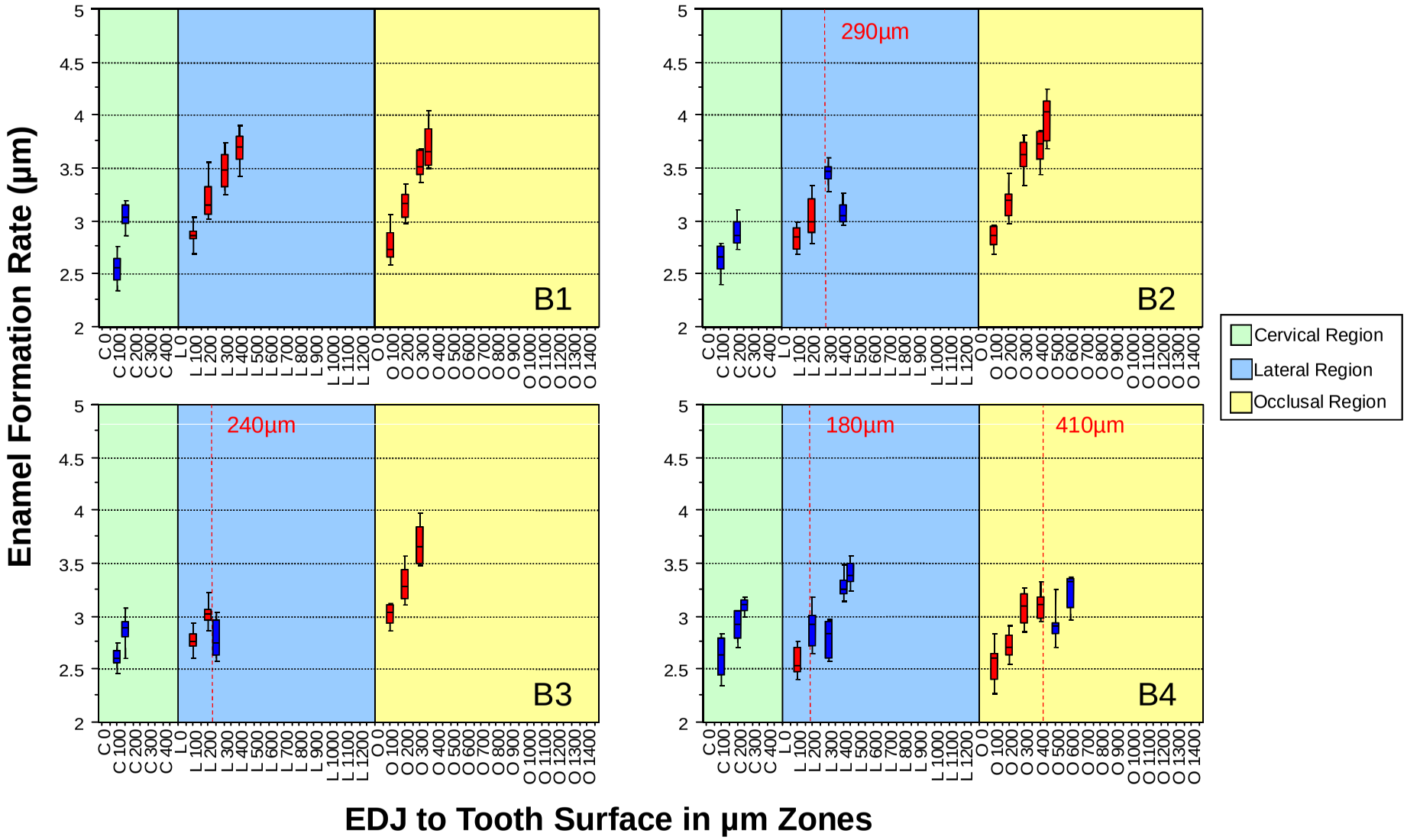


Figure 6.11: These box plots show the enamel formation rates for the labial aspect of four lateral incisors. Cervical, lateral and occlusal enamel regions are denoted by different shaded backgrounds. The vertical broken red line shows the position of the NNL with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

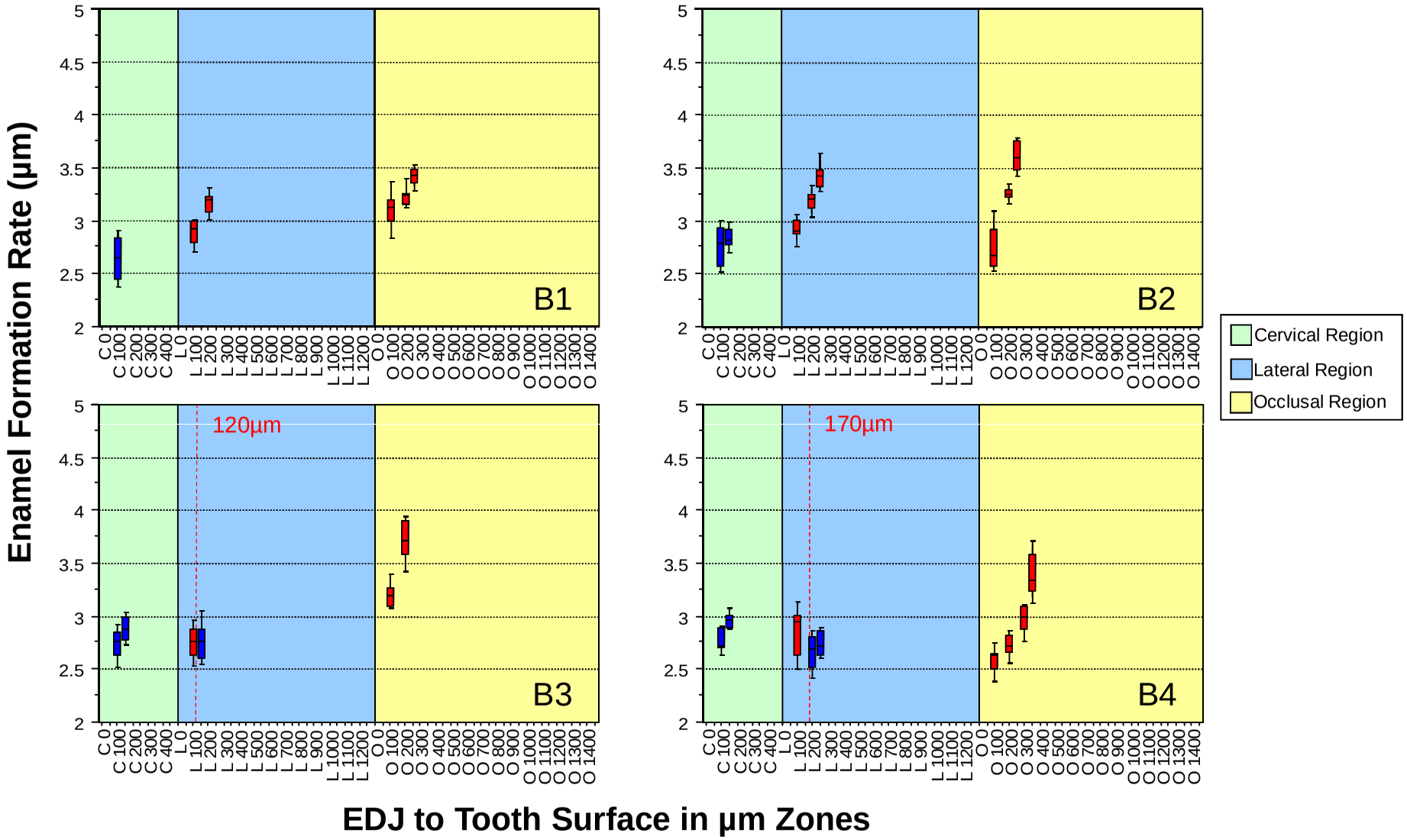


Figure 6.12: These box plots show the enamel formation rates for the lingual aspect of four lateral incisors. Cervical, lateral and occlusal enamel regions are denoted by different shaded backgrounds. The vertical broken red line shows the position of the NNL with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

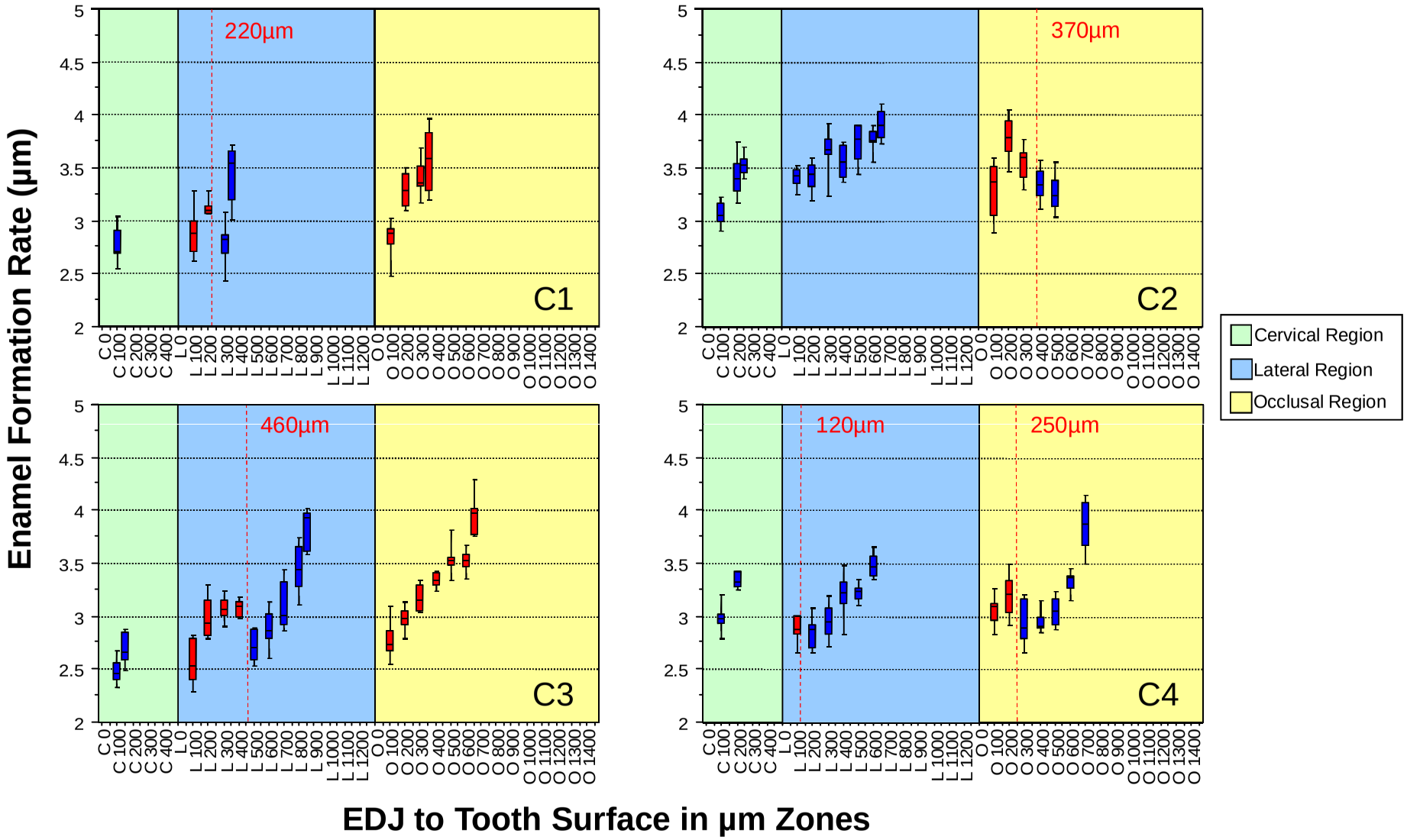


Figure 6.13: These box plots show the enamel formation rates for the labial aspect of four canines. Cervical, lateral and occlusal enamel regions are denoted by different shaded backgrounds. The vertical broken red line shows the position of the NNL with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

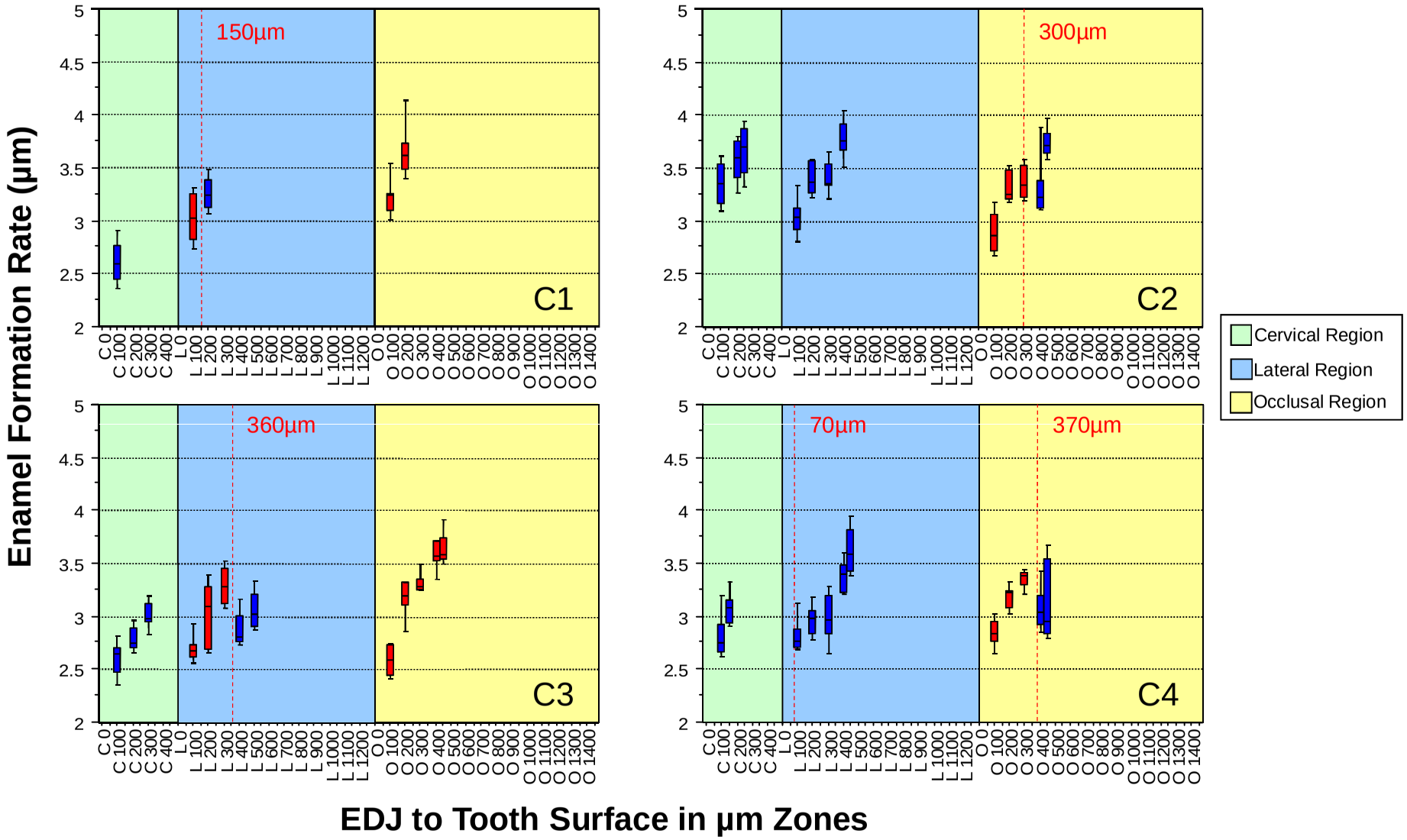


Figure 6.14: These box plots show the enamel formation rates for the lingual aspect of four canines. Cervical, lateral and occlusal enamel regions are denoted by different shaded backgrounds. The vertical broken red line shows the position of the NNL with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

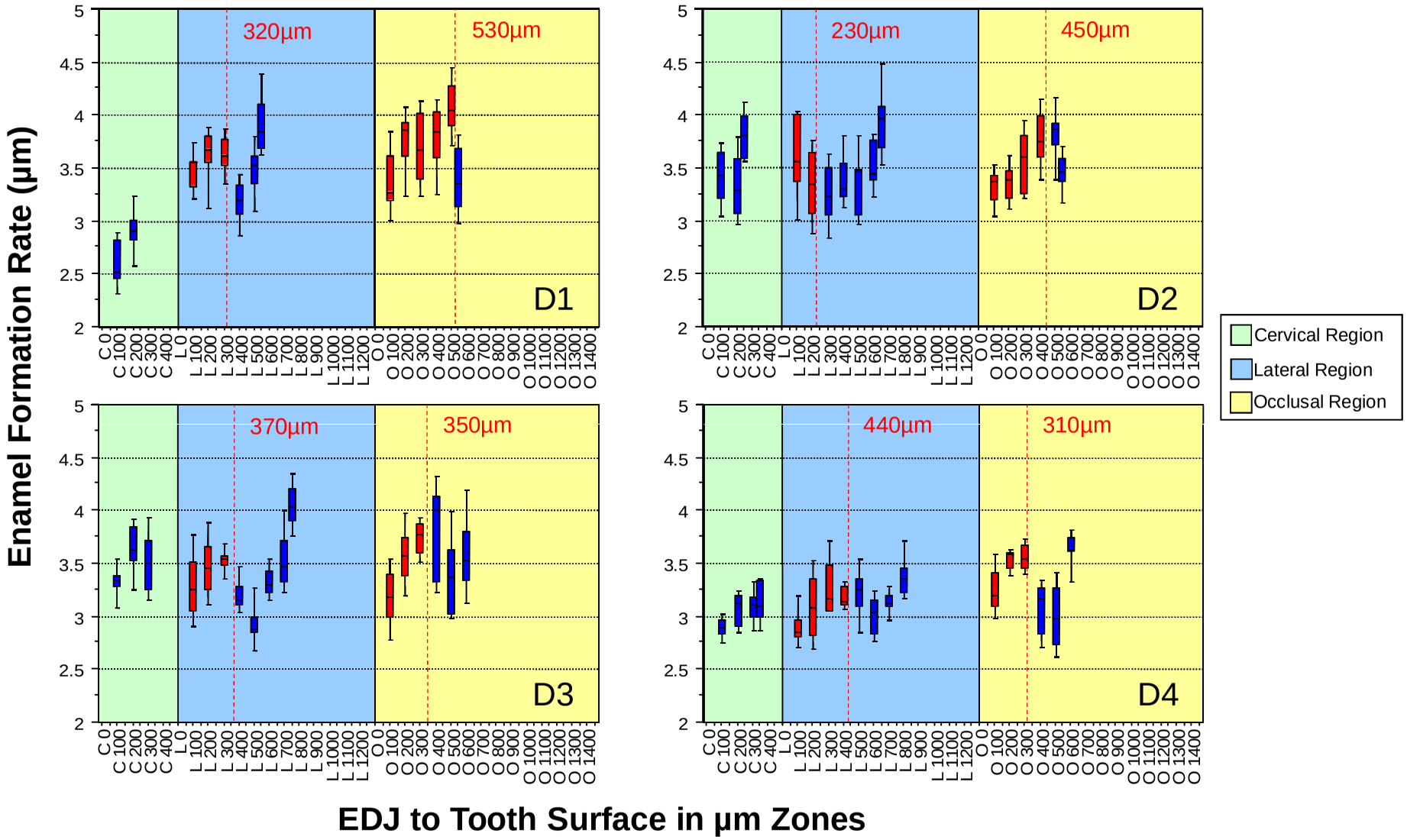


Figure 6.15: These box plots show the enamel formation rates for the buccal aspect of four first molars. Cervical, lateral and occlusal enamel regions are denoted by different shaded backgrounds. The vertical broken red line shows the position of the NNL with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

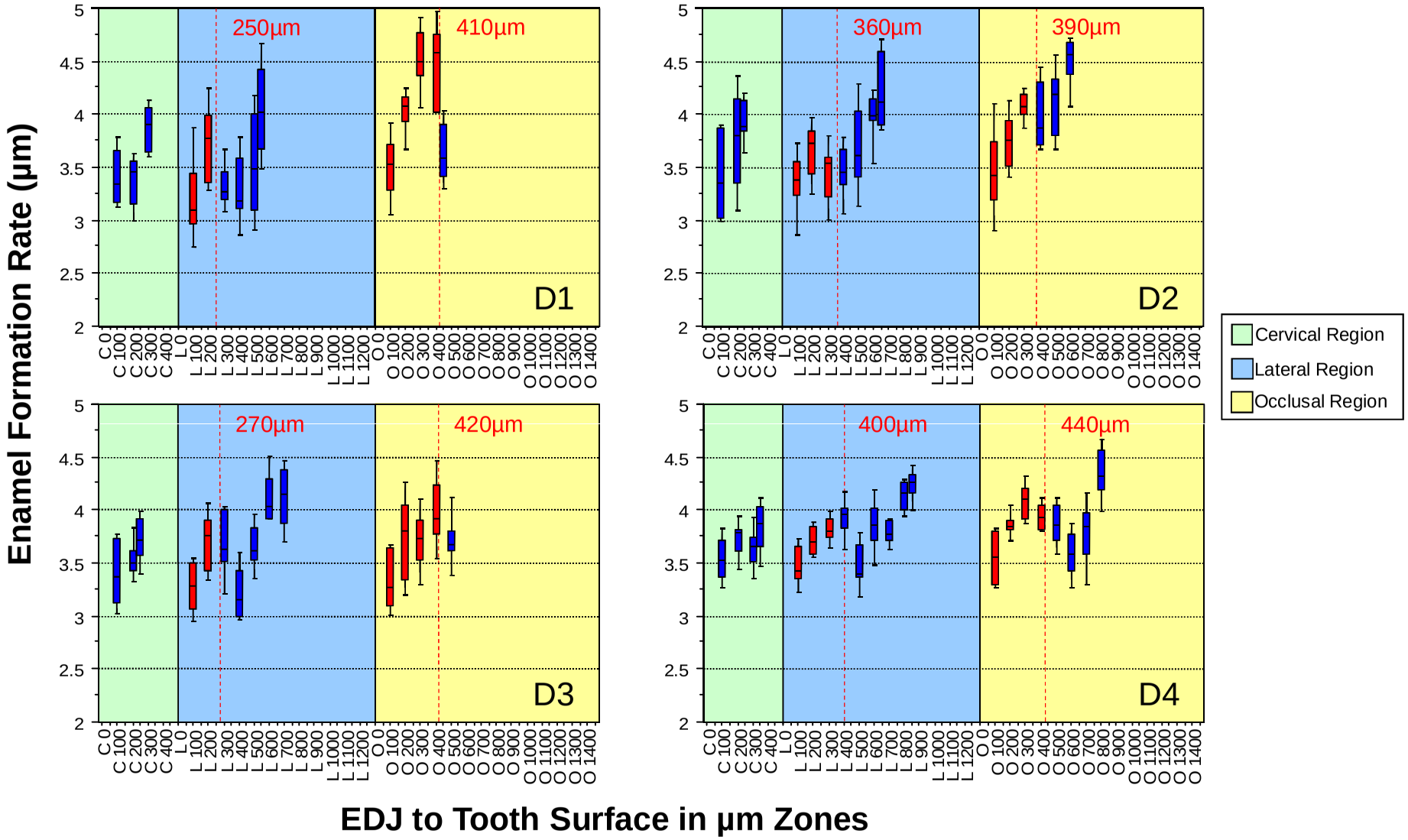


Figure 6.16: These box plots show the enamel formation rates for the lingual aspect of four first molars. Cervical, lateral and occlusal enamel regions are denoted by different shaded backgrounds. The vertical broken red line shows the position of the NNL with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

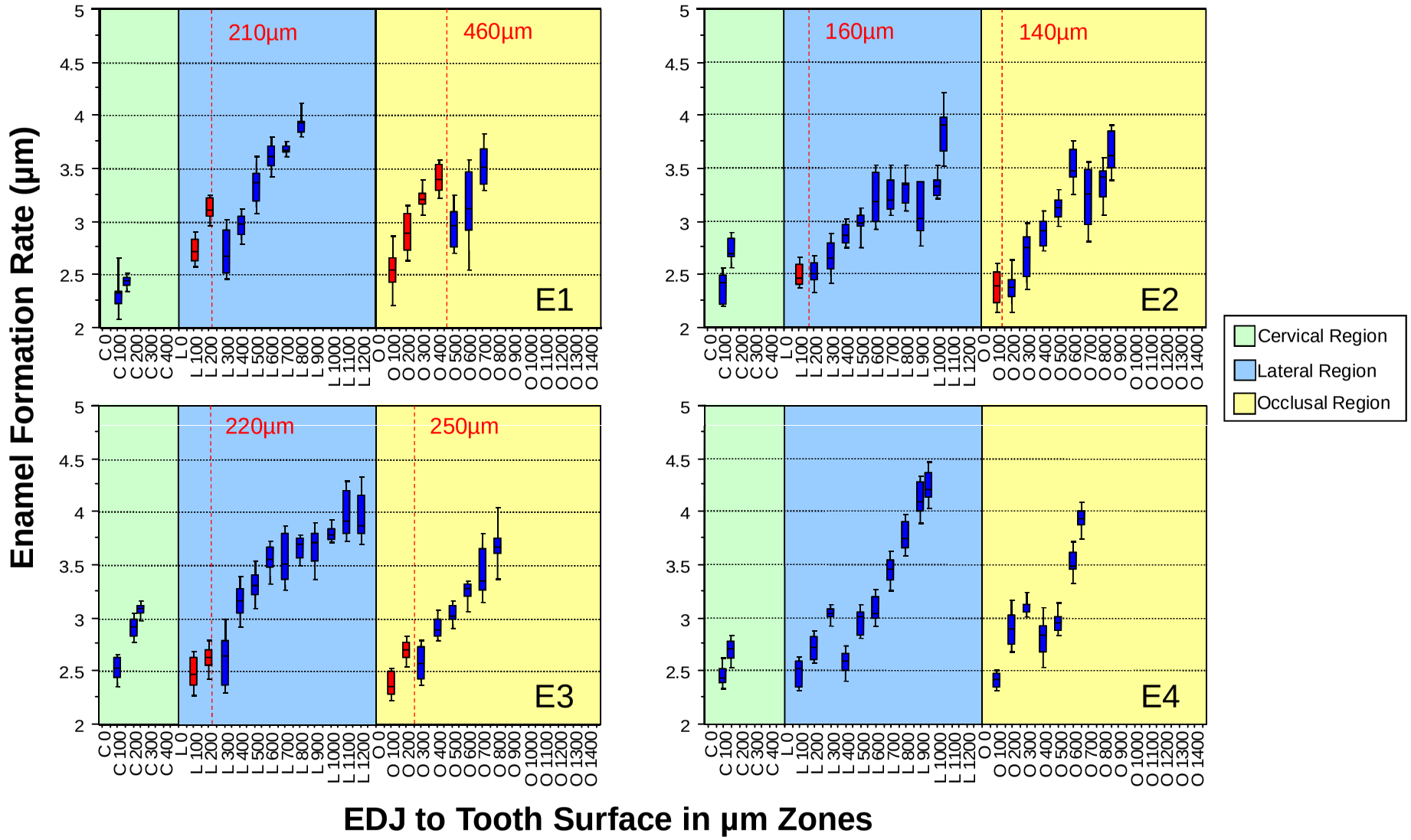


Figure 6.17: These box plots show the enamel formation rates for the buccal aspect of four second molars. Cervical, lateral and occlusal enamel regions are denoted by different shaded backgrounds. The vertical broken red line shows the position of the NNL with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

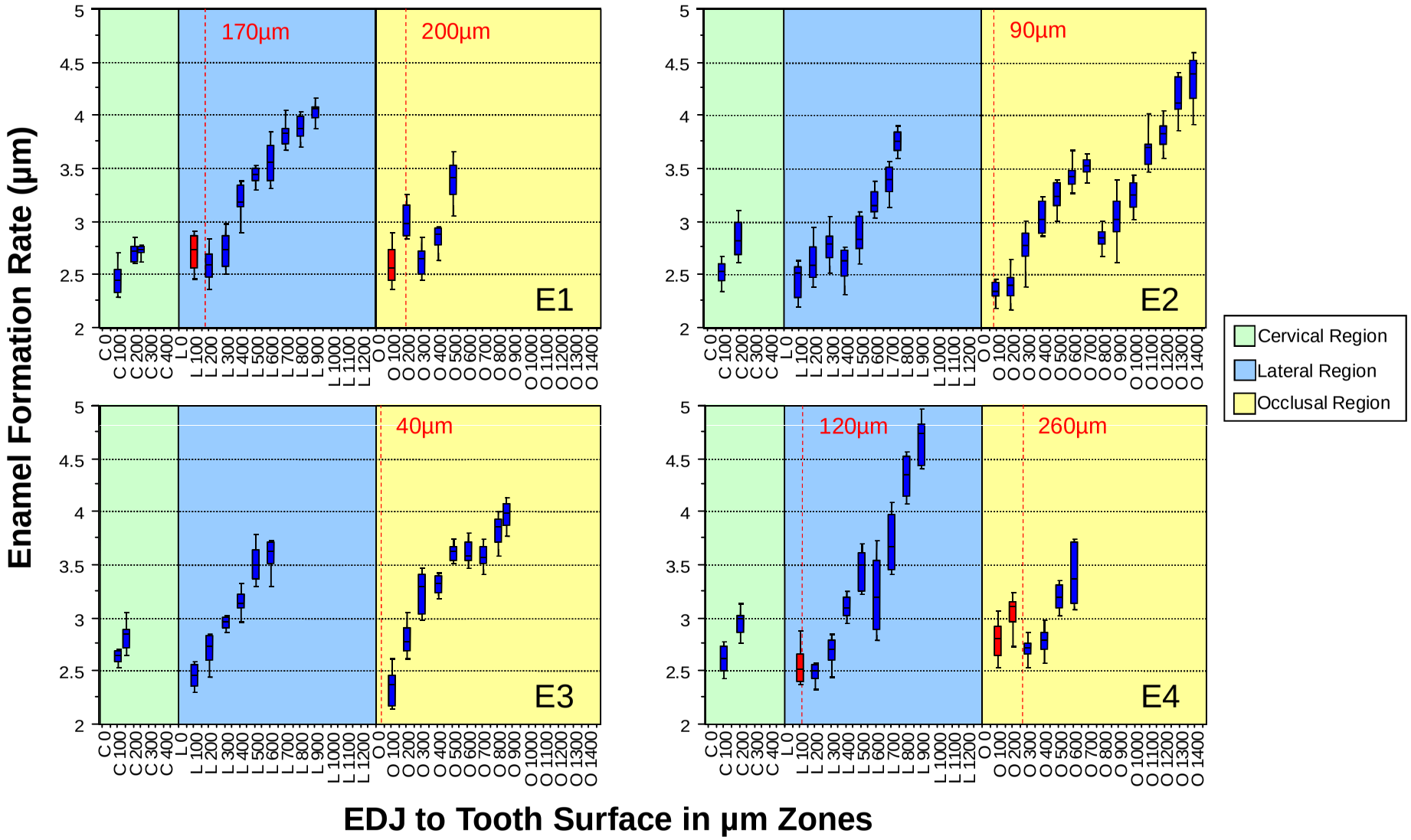


Figure 6.18: These box plots show the enamel formation rates for the lingual aspect of four second molars. Cervical, lateral and occlusal enamel regions are denoted by different shaded backgrounds. The vertical broken red line shows the position of the NNL with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

6.2.1 Summary Discussion

In general these box plots demonstrate an increase in the rate of enamel formation from the EDJ to the surface enamel; the rate of formation was slightly slower in the cervical enamel compared to the cuspal enamel. Daily increments ranged between a minimum value of 2.08µm at the EDJ to a maximum value of 4.22µm at the enamel surface in the cervical enamel, between 2.15µm at the EDJ to 4.97µm at the enamel surface in the lateral enamel and 2.07µm at the EDJ to 4.73µm at the enamel surface in the occlusal enamel (the extreme data points for each region in each tooth type are highlighted in **Appendix Three** and the data points mentioned above are shown in red).

The total weighted mean rates of enamel formation within a given 100µm box plot zone in cervical enamel ranged from 2.80µm at the EDJ to 3.06µm at the enamel surface, between 2.86µm at the EDJ to 3.51µm at the enamel surface in the lateral enamel and between 2.90µm at the EDJ to 3.64µm at the enamel surface in the occlusal enamel. These calculations are presented in **Appendix Four**.

Superimposed upon this general pattern there was an almost universal sharp decrease in the rate of enamel formation in the 100µm zone following either the neonatal line or certain other accentuated incremental markings. Generally, this decrease was initially of the order of up to 0.5µm per day. Within the subsequent 100µm zone, there was a catch-up phase where rates of enamel formation generally returned to their previous values within a 400µm period. This reduction in the enamel formation rate is clear evidence of enamel hypoplasia associated with stress lines in enamel. The hypoplastic phase and the hyperplastic catch-up phase generally occurred within a single 100µm zone of enamel thickness. The most distinct of these catch-up phases occurs just after birth. It is, evident however, that the presence of other pronounced striae, possibly caused by the stress of illness during early childhood, also seem to influence the rate of enamel formation.

Three teeth (C4, E2 and E4), all exhibited decreases in the enamel formation rate that did not appear to be related just to the presence of the neonatal line.

Upon further investigation of the ground section, ‘stress lines’ were observed and the position of these was plotted on the relevant graphs (**Figure 6.19 – 6.20**). In **Figure 6.20**, the lateral region of lingual enamel of tooth E4 indicates that the presence of the neonatal line and two further ‘stress lines’ (marked as different green broken lines) appear to have resulted in the rate of enamel formation decreasing on three separate occasions.

The neonatal line was not always present in the enamel areas examined, for example it was not present in the occlusal enamel of the central and lateral incisors, except for the labial aspect of tooth B4 (**Figure 6.11**), however here the neonatal line can be clearly seen to influence the enamel formation rate, while in the other incisor plots the enamel formation rate increases regularly with increasing prism length.

The delay in the decrease of the enamel formation rate after a neonatal line in some of the box plots may be explained by the fact that the neonatal line is sometimes only just included within a 100µm zone. An example of this can be seen in tooth B2 in **Figure 6.11** where the neonatal line occurs at 290µm from the EDJ, but the decrease in the rate of enamel formation is not demonstrated until the next 100µm zone. On other occasions the neonatal line may pass through a zone at an angle. In which case only part of the zone will then contain prenatal enamel and the rest will contain postnatal enamel and this will also influence how the box plot averages reflect the timing of decreased enamel formation rates.

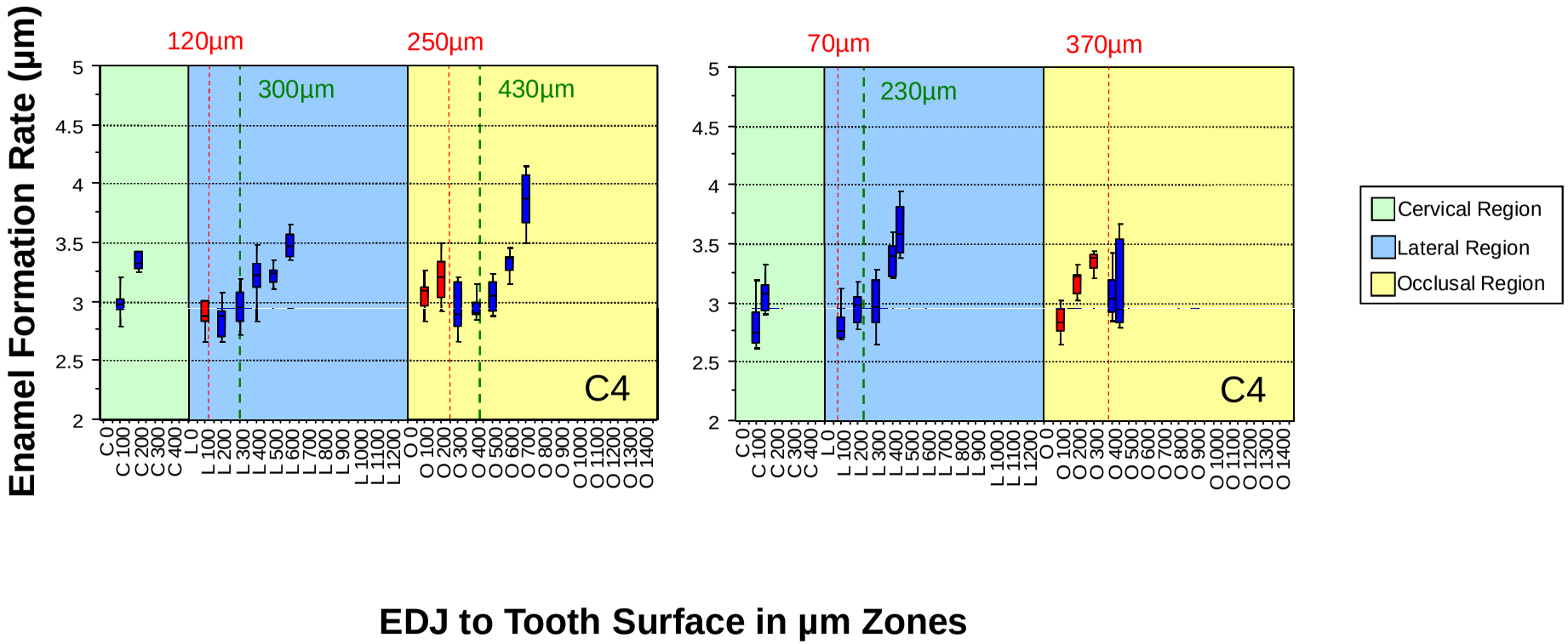


Figure 6.19: These box plots show the enamel formation rates for the labial aspect (on the left) and the lingual aspect (on the right) of one canine. As in the previous figures the cervical, lateral and occlusal enamel regions are denoted by different shaded backgrounds. The vertical broken red line shows the position of the NNL with respect to the EDJ while the vertical broken green line shows the position of a 'stress line' with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

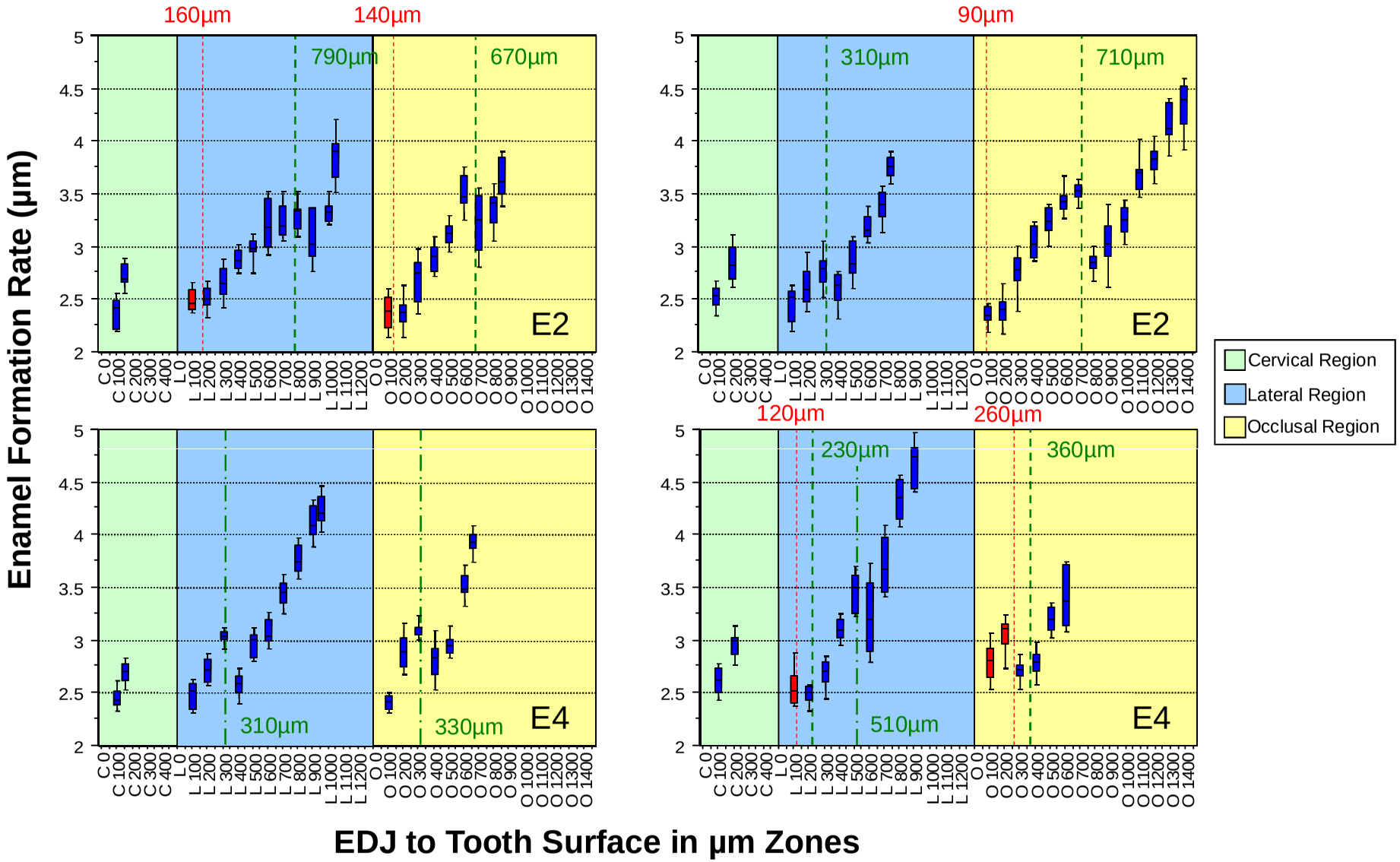


Figure 6.20: These box plots show the enamel formation rates for the buccal aspects (on the left) and the lingual aspects (on the right) of two second molars. As in the previous figures the vertical broken red line shows the position of the NNL with respect to the EDJ while the vertical broken green lines show the position of 'stress lines' with respect to the EDJ. Individual box plots for prenatal enamel are filled red and for postnatal enamel filled blue. Each box plot is for ten groups of six cross-striations in every 100µm zone of enamel measured from the EDJ. The horizontal lines show the 25th, 50th and 75th centiles, the whiskers indicate the 10th and 90th centiles.

CHAPTER 7: Application of Regression Formulae to Three Case Studies

7.1 Materials Methods and Data Collection

7.1.1 Materials

From a selection of longitudinal ground sections produced by Professor Christopher Dean from the deciduous teeth of three individuals (Twin A, Twin B and Individual C), three slides were selected. Mandibular teeth were again examined as these tend to be found as individual skeletal elements, both in an archaeological and forensic context, while maxillary teeth tend to remain in situ in the maxilla enabling other methods of age estimation such as the degree of dental eruption or suture closure etc. to be used in these situations. In addition as mandibular teeth have been used to generate the regression formulae it was hoped that more accurate results would be obtained by using similar teeth.

Initially second molars were selected as they exhibit the largest amount of enamel after birth and therefore potentially offer a longer time-line than the other deciduous teeth. However, as the regression equations did not permit very small distances of enamel to be substituted into the formulae, two first molars (Twin A and B) and one canine (Individual C) were selected in order to obtain as much postnatal information as possible.

Although some striae were clearer on the lingual aspects, the buccal aspect was examined as this aspect is thicker and so contains the greatest number of increments of growth from initiation at the EDJ until the end of enamel formation at the buccal cervix. Therefore in a forensic context the buccal aspect is of more use when trying to establish an estimated age of an individual, as it potentially offers a longer time-line than the lingual enamel. The buccal aspect was also shown to be more statistically constant during the analysis of the regression formulae (see **Section 6.1.3** and **6.1.5**).

The medical histories for these three individuals were also available and were consulted after the data had been obtained.

7.1.2 Methods

Production of Photomontages

Photomontages were produced of the ground sections from these additional individuals, Twin A, Twin B and Individual C. These montages were constructed from a series of overlapping photographic prints taken with an Olympus OM-2N camera loaded with Kodak Gold 200 film attached to a Carl Zeiss Janamed 2 light microscope with an apochromat 25x/0.65 ∞/0.17-A objective lens; from a 5 x 7 inch print of the negative the resultant field width was 410µm.

A sequence of photographs was taken along the course of the enamel prisms from the EDJ to the surface of the tooth, so that a complete record of the incremental growth of the enamel could be obtained. This record covers the first enamel formed next to the EDJ to the last layers of enamel formed at the tooth surface, i.e. that formed just before root formation began, or before the tooth was exfoliated. Photomontages were constructed of the occlusal region where the prenatal crown formation times had been previously calculated from in sample group one (see **Section 5.2.1.**) and also slightly lower in the occlusal region where the postnatal enamel exhibited the clearest accentuated striae. This process was repeated for each of the three individuals.

7.1.3 Data Collection

Once constructed, the photomontages were then examined in greater detail. The examination of each set of montages for each individual was completed in one session, so as to decrease the possibility of observer error within the ground section for each individual, in addition each individual was treated as a separate case rather than working on all three at the same time.

The Recording and Calculation of the Time Taken Between Each Accentuated Striae

In order to ascertain the time taken between each accentuated striae the methods used previously were adapted. Once the photomontages had been constructed, a sheet of clear acetate was placed over the photomontage and secured firmly in place with ‘Sellotape’. The positions of the neonatal line and any other accentuated striae were then traced onto the acetate using a fine-tipped permanent Staedtler Lumocolor pen. Care was taken to make constant reference to the ground section whilst this was being done in order to ensure that the striae were accurately identified on the photomontage and so correctly traced onto the acetate.

A straight line was then drawn onto the acetate running in the general prism direction; care was taken to position the line along the majority of the prism path from its beginning at the EDJ to its termination at the enamel surface. This line was placed in the same location as earlier, when calculating the prenatal enamel formation times (see **Section 5.3.2.a**). The distance from the EDJ to the location where each accentuated striae crossed this line was recorded, each time recording the prism length from the EDJ to the point where the line first encountered each accentuated stria. Each measurement was repeated three times and the mean was recorded on the acetate.

The mean distance measured from the EDJ to each subsequent accentuated striae was then converted from millimetres into micrometers. These resultant distances were then substituted into the appropriate linear regression formulae that had been derived from the cumulative counts of daily incremental cross-striations from sample group one (see **Section 5.3.1**). Prenatal and postnatal enamel formation times were calculated in this way for each of the buccal aspects for each of the three individuals.

Another clear acetate sheet was then placed on top of the acetate with the traced accentuated striae and secured in place with ‘Sellotape’. Where visible, daily cross-striation counts were recorded between each of the subsequent accentuated striae; commencing from the EDJ to the enamel surface following

the course of the straight line. Each cross-striation was carefully marked onto the acetate with a fine-tipped permanent Staedtler Lumocolor pen, therefore allowing this count to be verified and double checked. Where cross-striations were not always visible along the length of the same prism, the adjacent prism was used and the counts were transcribed onto this prism. When the use of a neighbouring prism was unavoidable, great care was taken not to add or subtract increments and so introduce errors into the final count. The number of counts of the daily increments at the point where the prism first encountered each accentuated striae was recorded.

The results obtained from the application of the regression formulae and the daily cross-striation counts were tabulated and then compared to each medical history.

7.2 Results

In order to ascertain the time taken between each accentuated striae, the regression formulae developed in the previous section, were applied to measurements taken from the photomontages. Daily cross-striation counts were also recorded.

7.2.1 Twins A and B

Prenatal Enamel

The results obtained for the prenatal formation times can be found in **Table 7.1** this includes the predicted times derived from the formulae and the corresponding daily cross-striation counts. The prenatal crown formation times for both twins calculated using the regression formulae resulted in 78 days of prenatal enamel formation for each individual; the daily cross-striation counts were very similar being 75 days for Twin A and 74 days for Twin B. Only on one occasion in Twin B did the mean formulae results and the daily counts match exactly (shown in black in **Table 7.1**). However, there is only a discrepancy of

four days maximum, between the mean formulae results and the daily cross-striation counts and on no occasion do the corresponding direct counts fall outside of the range of the 95% confidence limits.

These prenatal enamel formation times appear to be much shorter than the prenatal formation times calculated previously for first molars (see **Section 5.3.1** and **Table 6.4**). This suggests that these twins may have been born prematurely, if the prenatal enamel formation time (78 days) is subtracted from the first molar crown formation time previously calculated (140 days with a range of 135-146 days) then this infers that the twins were born 62 days prematurely.

The prenatal enamel found in both of these molars is also unlike the prenatal enamel that was examined previously; this enamel appears to contain accentuated striae approximately reoccurring every four to seven days until the neonatal line is encountered. On average for both twins this is every 5.95 days. All of these accentuated striae are similar in appearance and although faint, they are clearly visible. This is unusual as prenatal enamel usually forms regularly and consistently. In this case however, these lines may be indicative of maternal ill health (see Twin A and Twin B Health Histories below). From day 18-19 after mineralisation had commenced, the first stria is visible, from this time approximately every week another stria occurs until the neonatal line is encountered.

The number of these prenatal accentuated lines differs between the twins; there are 11 visible in Twin A while only nine are visible in Twin B. Even when the acetate from Twin A was placed over the corresponding region in Twin B and the ground section was consulted again, still only nine striae were visible in the section from Twin B. Although there are two less of these lines expressed in the prenatal enamel of Twin B, the occurrence of all of the other accentuated prenatal striae corresponds with those in the enamel of Twin A, it is possible that they are just not visible in this section. Furthermore, there is ‘potential space’ in the enamel where these two lines ‘would’ occur, if they were visible then their position would be at the equivalent level of 34 days and 59 days as they are in Twin A.

Table 7.1: This table shows the predicted mean results of the combined regression formulae when applied to the prenatal enamel of two ground sections of the first molar teeth from two individuals. The crown formation times are expressed in days, as well as indicating the error margin in days (rounded to the nearest day). The cross-striation counts for each corresponding measurement are also presented to allow direct comparison of the two methods. Black mean numbers correspond exactly between the mean regression formulae counts and the direct daily counts.

Twin A

Measurements From Photomontage

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days) Predicted	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Corresponding Cross-striation Direct Counts
A	27.22	2.24	60.95	19	16	21	18
B	36.16	2.24	81.00	24	21	27	23
C	46.35	2.24	103.82	30	27	33	28
D	53.92	2.24	120.78	34	31	37	32
E	63.32	2.24	141.84	39	36	43	38
F	71.87	2.24	160.99	44	41	47	43
G	80.47	2.24	180.25	49	46	52	48
H	89.51	2.24	200.50	54	51	58	52
I	97.49	2.24	218.38	59	55	62	56
J	105.32	2.24	235.92	63	59	67	62
K	114.27	2.24	255.96	68	64	72	66
L	131.15	2.24	293.78	78	74	82	75

Twin B

Measurements From Photomontage

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days) Predicted	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Corresponding Cross-striation Direct Counts
A	25.71	2.24	57.59	18	15	21	17
B	35.55	2.24	79.79	24	21	26	22
C	49.19	2.24	110.19	**31**	28	34	**31**
D	62.86	2.24	140.81	39	36	42	38
E	70.03	2.24	156.87	43	40	46	41
F	76.86	2.24	172.17	47	44	50	46
G	89.66	2.24	200.84	54	51	58	53
H	105.13	2.24	235.49	63	59	67	60
I	116.14	2.24	260.15	69	65	73	66
J	131.41	2.24	294.36	78	74	82	74

Mean
Enamel Formation Time (days) = 0.254 x Prism Length (µm) + 3.291
Lower 95% Confidence Limit
Enamel Formation Time (days) = 0.248 x Prism Length (µm) + 0.951
Upper 95% Confidence Limit
Enamel Formation Time (days) = 0.260 x Prism Length (µm) + 5.631

Postnatal Enamel

The results obtained for the postnatal formation times can be found in **Table 7.2** this includes the predicted times derived from the formulae and the daily cross-striation counts. The striae in the postnatal enamel are much less regularly spaced than those in the prenatal enamel and unlike the prenatal enamel some lines appear more pronounced than others. The postnatal formation times of the striae nearest to the enamel surface for both twins was calculated using the regression formulae and this resulted in 129 days of postnatal enamel formation for Twin A and a corresponding cross-striation count of 132 days and 126 days for Twin B with a corresponding cross-striation count of 120 days. Again the comparison of the results obtained for both twins was similar. The results obtained by the use of the regression formulae and the daily counts were also similar for both twins, for this 'final' stria for Twin A there was a discrepancy of three days between the formulae and the direct daily counts and for Twin B there was a discrepancy of six days, however this still fell within the 120-131 day 95% confidence limit range. On five occasions in Twin A and seven in Twin B the mean formulae results and the daily counts matched exactly (shown in black in **Table 7.2**). Again on no occasion do the corresponding direct counts fall outside of the range of the 95% confidence limits.

Comparison of the Photomontages with the Known Medical History

The results obtained using the regression formulae for the both twins can be found in **Table 7.3**. The results obtained using the formulae were then compared to the medical history, which are included below. Days when a direct comparison could be made between the medical history and the striae locations are highlighted in green in **Table 7.3**.

Table 7.2: This table shows the predicted mean results of the combined regression formulae when applied to the postnatal enamel of two ground sections of the first molar teeth from two individuals. The crown formation times are expressed in days, as well as indicating the error margin in days (rounded to the nearest day). The cross-striation counts for each corresponding measurement are also presented to allow direct comparison of the two methods. Black mean numbers correspond exactly between the mean regression formulae counts and the direct daily counts.

Twin A

Measurements From Photomontage

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days) Predicted	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Corresponding Cross-striation Direct
A	8.16	2.24	18.32	8	5	10	7
B	21.26	2.24	47.62	**15**	13	18	**15**
C	29.48	2.24	66.04	**20**	17	23	**20**
D	32.81	2.24	73.49	**22**	19	25	**22**
E	36.33	2.24	81.38	**24**	21	27	**24**
F	41.64	2.24	93.27	**27**	24	30	**27**
G	66.66	2.24	149.32	41	38	44	43
H	80.35	2.24	179.98	49	46	52	52
I	85.95	2.24	192.53	52	49	56	56
J	93.26	2.24	208.90	56	53	60	60
K	97.87	2.24	219.23	59	55	63	62
L	103.75	2.24	232.40	62	59	66	66
M	111.55	2.24	249.87	67	63	71	70
N	121.31	2.24	271.73	72	68	76	74
O	142.38	2.24	318.93	84	80	89	86
P	163.06	2.24	365.25	96	92	101	98
Q	195.27	2.24	437.40	114	109	119	116
R	221.1	2.24	495.26	129	124	134	132

Twin B

Measurements From Photomontage

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days) Predicted	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Corresponding Cross-striation Direct
A	8.98	2.24	20.16	8	6	11	7
B	14.91	2.24	33.40	12	9	14	11
C	30.02	2.24	67.24	**20**	18	23	**20**
D	33.74	2.24	75.58	**22**	20	25	**22**
E	44.02	2.24	98.60	**28**	25	31	**28**
F	51.12	2.24	114.51	**32**	29	35	**32**
G	56.44	2.24	126.43	**35**	32	39	**35**
H	59.3	2.24	132.83	**37**	34	40	**37**
I	86.71	2.24	194.23	53	49	56	50
J	91.42	2.24	204.78	55	52	59	54
K	96.61	2.24	216.41	58	55	62	57
L	102.49	2.24	229.58	62	58	65	61
M	107.65	2.24	241.14	65	61	68	64
N	113.13	2.24	253.41	68	64	72	67
O	120.1	2.24	269.02	72	68	76	71
P	156.35	2.24	350.22	**92**	88	97	**92**
Q	162.15	2.24	363.22	96	91	100	95
R	215.02	2.24	481.64	126	120	131	120

Mean
Enamel Formation Time (days) = 0.254 x Prism Length (µm) + 3.291
Lower 95% Confidence Limit
Enamel Formation Time (days) = 0.248 x Prism Length (µm) + 0.951
Upper 95% Confidence Limit
Enamel Formation Time (days) = 0.260 x Prism Length (µm) + 5.631

Table 7.3: This table shows the predicted mean results of the combined regression formulae for both prenatal (red) and postnatal (blue) enamel of two ground sections of the first molar teeth from two individuals. The crown formation times are expressed in days, as well as indicating the error margin in days (rounded to the nearest day). The green highlighted numbers correspond to points in the medical histories. Black mean numbers correspond exactly between Twin A and Twin B.

Twin A

Measurements From Photomontage			Confidence Limits	
Measurement	Prism Length (µm)	Mean (days) Predicted	95% Lower (days)	95% Upper (days)
A	60.95	19	16	21
B	81.00	**24**	21	27
C	103.82	30	27	33
D	120.78	34	31	37
E	141.84	**39**	36	43
F	160.99	44	41	47
G	180.25	49	46	52
H	200.50	**54**	51	58
I	218.38	59	55	62
J	235.92	**63**	59	67
K	255.96	68	64	72
L	293.78	**78**	74	82
A	18.32	**8**	5	10
B	47.62	15	13	18
C	66.04	**20**	17	23
D	73.49	**22**	19	25
E	81.38	24	21	27
F	93.27	27	24	30
G	149.32	41	38	44
H	179.98	49	46	52
I	192.53	52	49	56
J	208.90	56	53	60
K	219.23	59	55	63
L	232.40	**62**	59	66
M	249.87	67	63	71
N	271.73	**72**	68	76
O	318.93	84	80	89
P	365.25	**96**	92	101
Q	437.40	114	109	119
R	495.26	129	124	134

Twin B

Measurements From Photomontage			Confidence Limits	
Measurement	Prism Length (µm)	Mean (days) Predicted	95% Lower (days)	95% Upper (days)
A	57.59	18	15	21
B	79.79	**24**	21	26
C	110.19	31	28	34
D	140.81	**39**	36	42
E	156.87	43	40	46
F	172.17	47	44	50
G	200.84	**54**	51	58
H	235.49	**63**	59	67
I	260.15	69	65	73
J	294.36	**78**	74	82
A	20.16	**8**	6	11
B	33.40	12	9	14
C	67.24	**20**	18	23
D	75.58	**22**	20	25
E	98.60	28	25	31
F	114.51	32	29	35
G	126.43	35	32	39
H	132.83	37	34	40
I	194.23	53	49	56
J	204.78	55	52	59
K	216.41	58	55	62
L	229.58	**62**	58	65
M	241.14	65	61	68
N	253.41	68	64	72
O	269.02	**72**	68	76
P	350.22	92	88	97
Q	363.22	**96**	91	100
R	481.64	126	120	131

The medical history revealed after the histological analysis was completed is included below.

Twin A and Twin B Health Histories

Male twins were born by emergency C-section about 6pm (counted as day zero)*. At a doctor's visit earlier that day, Baby B was judged to be in distress. Twins were 32 weeks gestation.*

Baby B's growth had flat-lined since about 30 weeks as measured on ultrasound. At birth Baby B was small, only 956gm and his placenta was small. The reason was presumed to be anti-phospholipid antibody syndrome (a clotting disorder of particular significance in pregnancy)*, for which the mother was treated throughout pregnancy with heparin and aspirin. Weekly steroid injections were also administered three weeks before birth, in order to 'pull the twins' lung development along a little faster'. Babies are both A+ the mother is O+. Baby A was a reasonable size for date, born at 1765gm.*

Feeding diaries span days 26 to about 200; after that the babies are more robust, are eating some solid food and the diaries stop.

Events Shared

Day 0: *Birth. Babies were put on a ventilator overnight the first night but taken off sometime on Day 1. Both kept in NICU (neonatal intensive care unit) at a major university hospital. They were judged to be doing well and were quickly graduated from rooms 1-3* (graded in intensity of care).

Day 7: *Hepatitis B immunisation.*

Day 12: *Transferred by ambulance to a different hospital with a step-down special care unit because they were too well to stay in the NICU.*

Day 24/26: *Baby A home from hospital on day 24; Baby B home on day 26.*

Note: *Day 58 was their predicted due date for a 40 week gestation.*

Day 72: *DPT, Hepatitis B, H influenza type B and Polio.*

Day 72-76: *Both babies were given Tylenol* (acetaminophen) *over five days* (notes don't say why, but it suggests fever or indication of pain or discomfort following immunizations).

Day 126: *DPT, H influenza type B and Polio IPV immunisations. Both babies get Tylenol that day; Baby B gets Tylenol the next day also.*

Day 191: *DPT, H influenza type B immunisations. Both babies get Tylenol that day*

Extra Events for Baby B

Day 35-40: *GI upset on days 35-36; notes say he is also receiving eye medicine (days 35-42) and on day 40 is very fussy.*

Day 56-59: *Take him in to the Emergency Room late on day 56; he has surgery for intestine trapped hernia on day 57; he remains ill – vomiting through day 58, receiving Tylenol through day 59.*

DPT is a combination of vaccines which immunise against diphtheria, pertussis (whooping cough) and tetanus. The vaccine component includes diphtheria and tetanus toxoids and killed whole cells of the organism that causes pertussis.

Tylenol is also known as paracetamol in the UK.

A direct comparison between the timing of the occurrence of the accentuated striae and the corresponding events from the medical history can be found in **Table 7.4**.

Table 7.4: This table shows the predicted mean results of the combined regression formulae for the postnatal (blue) enamel of two ground sections of the first molar teeth from two individuals. The crown formation times are expressed in days, as well as indicating the error margin in days (rounded to the nearest day). The cross-striation counts for each corresponding measurement are also presented to allow direct comparison of the two methods. The green highlighted numbers correspond to points in the medical histories and the black mean regression formulae counts and cross-striation counts correspond exactly with the medical histories.

Twin A		Confidence Limits					Twin B		Confidence Limits				
Measurement	Mean (days) Predicted	95% Lower (days)	95% Upper (days)	Corresponding Cross-striation Direct Counts	Corresponding Medical History	Day	Measurement	Mean (days) Predicted	95% Lower (days)	95% Upper (days)	Corresponding Cross-striation Direct Counts	Corresponding Medical History	Day
					Birth placed on ventilator	0						Birth placed on ventilator	0
					Removed from ventilator	1						Removed from ventilator	1
A	8	5	10	**7**	Hepatitis B immunisation	**7**	A	8	6	11	**7**	Hepatitis B immunisation	**7**
B	15	13	18	15	Transfer from NICU to step-down unit	12	B	**12**	9	14	11	Transfer from NICU to step-down unit	**12**
C	20	17	23	20			C	20	18	23	20		
D	22	19	25	22			D	22	20	25	22	Discharged from hospital	26
E	**24**	21	27	**24**	Discharged from hospital	**24**							
F	**27**	24	30	**27**			E	28	25	31	28		
							F	32	29	35	32		
							G	**35**	32	39	35	Gastro-intestinal problems	**35**-36
							H	37	34	40	37	Eye problems	35-42
G	41	38	44	43									
H	49	46	52	52									
I	52	49	56	56			I	53	49	56	50		
J	56	53	60	60			J	55	52	59	54	Admitted to A and E	56-59
												Surgery for hernia	57
K	59	55	63	62			K	**58**	55	62	57	Vomiting	**58**
L	62	59	66	66			L	62	58	65	61	Received Tylenol	59
							M	65	61	68	64		
M	67	63	71	70			N	68	64	72	67		
N	**72**	68	76	74	DPT, Hepatitis B, H influenza type B, Polio	**72**	O	**72**	68	76	71	DPT, Hepatitis B, H influenza type B, Polio	**72**
					Received Tylenol	72-76						Received Tylenol	72-76
O	84	80	89	86			P	92	88	97	92		
P	96	92	101	98			Q	96	91	100	95		
Q	114	109	119	116									
R	129	124	134	132	DPT, H influenza type B, Polio and Tylenol	126	R	**126**	120	131	120	DPT, H influenza type B, Polio and Tylenol	**126**

Summary Discussion

From the initial observation of the ground sections is was apparent that Twin B had undergone more severe postnatal stress than Twin A and on closer observation of the photomontages this did indeed turn out to be the case. This was further collaborated by consultation with the medical history.

The prediction of the twin's premature birth which was calculated using the crown formation times derived from the regression formulae for first molars (see **Table 6.4**) was correct; however, the predicted time of 62 days was inaccurate by six days. Birth occurred after 32 weeks of an expected 40 week gestation making the twins eight weeks (56 days) premature, which results in a discrepancy of six days.

The three separate occasions that each twin was given an immunisation/vaccination injection can be clearly identified on the photomontages. For Twin A using the time derived from the formulae for the first injection (day 7) there is a discrepancy of one day, while the cross-striation counts corresponds exactly with the medical history. For the second injection (day 72) the formulae derived time corresponds exactly and the daily count is inconsistent by two days. For the third injection (day 126) there is a three day discrepancy with the formulae derived time and six days with the daily cross-striation counts. In Twin B using the time derived from the formulae for the first injection (day 7) there is a discrepancy of one day, while the cross-striation counts corresponds exactly with the medical history. For the second injection (day 72) the formulae derived time corresponds exactly and the daily count is inconsistent by one day. For the third injection (day 126) the formulae derived time corresponds exactly and there is a discrepancy of six days with the daily cross-striation counts.

What is very apparent from this study is that there are many incremental disturbances that can be identified in the developing enamel that are not recorded in the medical notes.

7.2.2 Individual C

Prenatal Enamel

Unlike the twins, the prenatal enamel from Individual C did not exhibit as many accentuated prenatal striae, there were three that were visible in the enamel in this area. The results obtained for the prenatal formation times can be found in **Table 7.5** this includes the predicted times derived from the formulae and the daily cross-striation counts. The prenatal crown formation time for Individual C calculated using the regression formulae resulted in 138 days of prenatal enamel formation; the daily cross-striation count was identical. Only on this one occasion did the prenatal mean formulae results and the daily cross-striation counts match exactly (shown in black in **Table 7.5**). However, on no occasion do the corresponding direct counts fall outside of the range of the 95% confidence limits.

The 138 days of prenatal enamel formation in Individual C is nearly similar to that calculated previously for canines (see **Section 5.3.1** and **Table 6.4**). The time derived from the regression formulae gives an average of 128 days prenatal enamel formation time with a range from 121 to 135 days. In the case of Individual C there is a discrepancy between the average times of ten days.

Postnatal Enamel

The results obtained for the postnatal formation times can be found in **Table 7.5**, this includes the predicted times derived from the formulae and the daily cross-striation counts.

In the postnatal enamel there are seven accentuated striae, three of which are very thin and bright and appear to be evenly spaced throughout the thickness of the postnatal enamel and another which although being bright appears to be more diffuse than the other three.

Table 7.5: This table shows the predicted mean results of the combined regression formulae when applied to the prenatal (red) and postnatal (blue) enamel of one ground section of the canine from one individual. The crown formation times are expressed in days, as well as indicating the error margin in days (rounded to the nearest day). The cross-striation counts for each corresponding measurement are also presented to allow direct comparison of the two methods. Black mean numbers correspond exactly between the mean regression formulae counts and the direct daily counts.

Individual C

Measurements From Photomontage

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days) Predicted	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Corresponding Cross-striation Direct Counts
A	92.74	2.26	209.59	60	55	65	58
B	108.22	2.26	244.58	69	64	74	68
C	178.85	2.26	404.20	109	103	116	105
D	229.16	2.26	517.90	**138**	130	145	**138**

Measurements From Photomontage

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days) Predicted	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Corresponding Cross-striation Direct Counts
A	5.96	2.26	13.47	11	7	14	5*
B	10.66	2.26	24.09	13	10	16	8*
C	74.93	2.26	169.34	**50**	45	54	**50**
D	154.07	2.26	348.20	95	89	101	97
E	228.29	2.26	515.94	138	130	145	131
F	290.29	2.26	656.06	173	164	181	159*
G	320.54	2.26	724.42	190	181	199	170 *

Mean
Enamel Formation Time (days) = 0.253 x Prism Length (µm) + 7.106
Lower 95% Confidence Limit
Enamel Formation Time (days) = 0.244 x Prism Length (µm) + 4.123
Upper 95% Confidence Limit
Enamel Formation Time (days) = 0.261 x Prism Length (µm) + 10.089

* The cross-striations were very unclear and difficult to see in these locations

The daily cross-striation counts for the first two accentuated striae after the neonatal line were difficult to see and were quite unclear, in these two regions the daily cross-striation counts fall outside the range of the 95% confidence limits derived from the regression formulae, however the third daily count matches the result produced by the regression formulae exactly (50 days). Although the next two counts both fall within the range of the 95% confidence limits, the last two counts fall outside of the 95% range, again in these last two areas the cross-striations were hard to identify.

The postnatal formation time of the stria nearest to the enamel surface for Individual C was calculated using the regression formulae and this resulted in 190 days of postnatal enamel formation and a corresponding cross-striation count of 170 days.

Unlike the twins the results obtained from the regression formulae and the daily cross-striation counts are not a good match, however this may be due to the fact that in four of these seven areas, the cross-striations were difficult to see. As one count corresponded exactly it seems reasonable to suggest that one possibility for this discrepancy is due to observer error and the difficulties experienced when taking these counts from enamel with poorly visible cross-striations, rather than being due to the use of the regression equations.

Comparison of the Photomontages with the Known Medical History

The results obtained using the formulae for Individual C can be found in **Table 7.6**. The results obtained using the formulae were then compared to the medical history. Days when a comparison could be made between the medical history and the striae locations are highlighted in green in **Table 7.6**.

The medical history that was revealed after the histological analysis was completed is included below.

Table 7.6: This table shows the predicted mean results of the combined regression formulae for postnatal enamel of one ground section of the canine from one individual. The crown formation times are expressed in days, as well as indicating the error margin in days (rounded to the nearest day). The cross-striation counts are also presented to allow direct comparison of the two methods. The green highlighted numbers correspond (loosely) with the medical history.

Individual C		Confidence Limits				
Measurement	**Mean (days) Predicted**	**95% Lower (days)**	**95% Upper (days)**	**Corresponding Cross-striation Direct Counts**	**Corresponding Medical History**	**Day**
					Birth	0
					First jab	2
A	11	7	14	5*		
B	13	10	16	8*		
C	50	45	54	50	Second jab	47
					Third jab	78
D	95	89	101	97	Fouth jab	106
E	138	130	145	131	GI upset with fever	134-155
F	173	164	181	159*	Malaria injection	155
G	190	181	199	170 *		

* The cross-striations were very unclear and difficult to see in these locations

Individual C Health History

Male D.O.B. 2.12.89 – oral history taken from mother December 2009

Note: *no written records exist (these have been lost quite recently apparently) and the mother does not read or write well but recalls her first-born sons medical history from memory.*

Mother lived in Western Kenya close to Lake Victoria with her parents. Mother became ill 5-6 months into pregnancy (she says that it happened with all her 4 children 'C' being the first and describes it as "malaria". Mother says that she was given "pills" by the doctor for this.

Later into pregnancy the mother had to leave the family home and came to live with a brother in Nairobi several hundred miles from the family home. She says her health improved but it was emotionally stressful because 'Cs' father had disappeared.

'C' was born on December 2nd 1989 and was nursed and breast fed by his mother all the time. The mother explained that in Kenya infants usually have to attend antenatal clinics on the same date of each month as their baby's birth date. But Christmas and New Year vacation may have delayed first visit to clinic until 18th January. The following dates are what the mother remembers ~20 year later.

2 days after birth – given first jab in the forearm
Mother says 2nd jab given in left thigh on January 18th 1990
Mother says 3rd jab given in right thigh on February 18th 1990
Mother says 4th jab given in gluts on March 18th 1990

Mother and baby were sent "up country" to look for the father at Easter 1990 but after 3 weeks or so 'C' became "very hot and very ill". He was taken to hospital and given an injection and a course of pills for "malaria".

'C' had 2 or 3 bouts of GI upsets and diarrhoea while up country but not a "constant upset" – mother thinks he may have had recurrent fevers.

'Jabs' were DPT vaccinations.

Summary Discussion

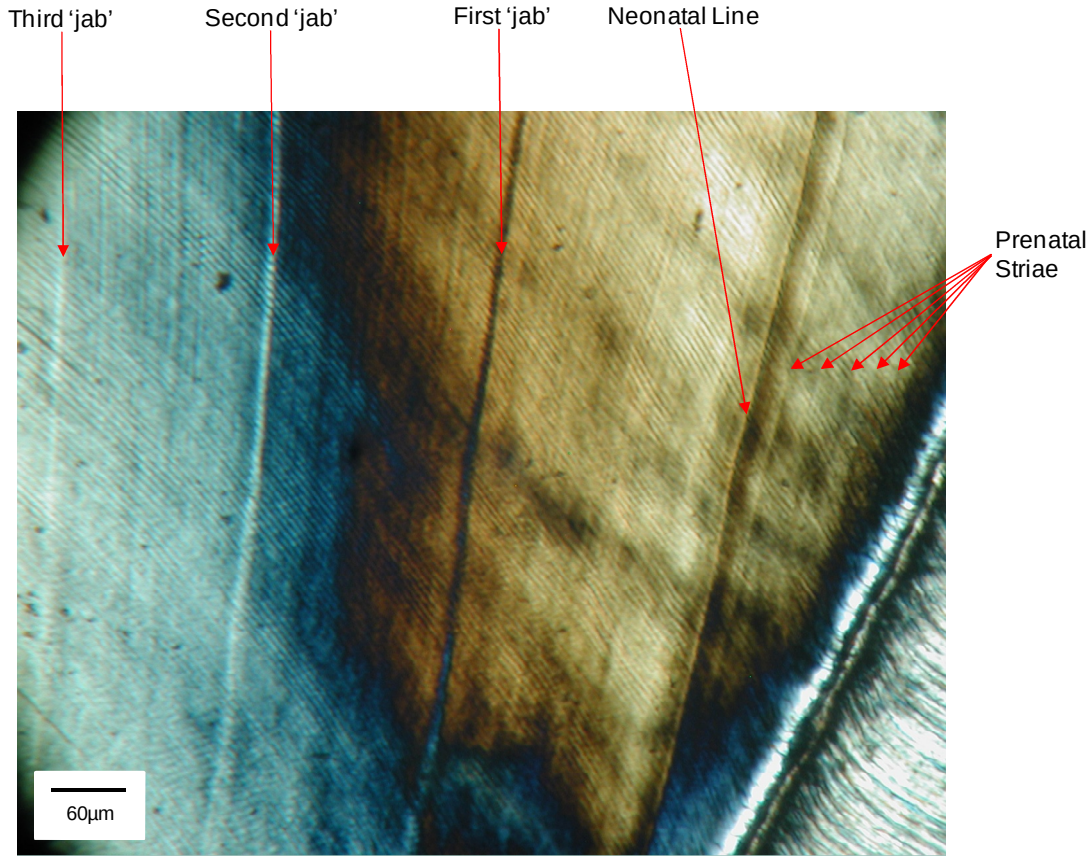
Unfortunately unlike the twins none of the dates from the medical history match exactly with the resultant calculations or counts.

It is possible that the accentuated striae in the prenatal enamel could refer to the "malaria" that the mother reported to have suffered from five to six months

into pregnancy, which would be three to four months before birth, this may be equivalent to the accentuated stria at day 109 (3.5 months).

In the postnatal enamel three very bright and distinct striae are visible and these are most probably related to the '*jabs*' that were given, however the medical history does not correspond with the time produced by either the regression formulae or the daily cross-striation counts (see **Table 7.6** and **Figure 7.1**).

Figure 7.1: This figure shows an example of the neonatal line, three 'jab' lines and prenatal striae from Individual C.



As the first '*jab*' was given on day two, the appearance of a corresponding line in the enamel may have been masked by the presence of the neonatal line, it may also have contributed to the difficulty in obtaining a clear daily cross-striation count in this area. With regards to the second '*jab*' given on the 18th January 1990 (47 days after birth), although there is no exact match for this date, this date does fall within the 95% confidence limit for the accentuated stria occurring at day 50 (range 45-54 days), so it is possible that this third accentuated stria corresponds to this date. The third '*jab*' was given on the 18th

February 1990 (78 days after birth) there are no accentuated striae corresponding to this date. The fourth '*jab*' was given on 18th March 1990 (106 days after birth) again there is no exact match for this date and it does not fall within the 95% limits of the next accentuated stria which occurs at day 95 (range 89-101 days). Although the dates do not correspond with the medical history there are three very similar lines of the same thickness and brightness that may be associated with these '*jabs*'. These three similar lines occur at days 50, 95 and 138 and could correspond to the modern UK DPT vaccination schedule, which is offered at eight weeks (56 days), 12 weeks (84 days) and 16 weeks (112 days) (National Health Service 2011a).

Three weeks (or so) after the Easter of 1990 (15th April) C became very ill and was treated for "malaria" in hospital with an injection and a course of tablets, this would have been about 134 days after birth, in addition, whilst "up country" C also suffered from two or three bouts of gastrointestinal disorder and may have had recurrent fevers, the possible dates for this could be 134 to 155 days after birth (calculated from Easter plus three weeks), it is possible that the fifth accentuated stria with a 95% range of 130 to 145 days could be related to this incident. Although Easter has been taken as Easter Sunday in this instance Easter may also be regarded as occurring the week before and after Easter Sunday, which does allow some flexibility with these dates.

Although there are seven postnatal accentuated striae, only two of these can be correlated to the medical history, these being the date of the second '*jab*' and the bout of gastrointestinal upset, which was accompanied by fever. As with the twins there are lines, which do not appear to be related to the medical history.

What is interesting in this case study is that the mother was able to recall Individual C's medical history to the day twenty years later. The clinician who took the history was aware of the eagerness to please on the part of many patients and relatives by perhaps trying hard to recall dates and events with presumed accuracy. So he tried to avoid excess questioning about key events.

7.3 Summary Discussion

Despite the inevitable nature of all but the most careful of written medical histories, the results of the histological analysis of Twin A and Twin B, together with their medical histories, provide considerable support for the usefulness and accuracy of the regression equations derived in **Section 6.1.6**.

The appearance of the immunisation/vaccination lines in the postnatal enamel of the twins are very similar to those '*jab*' lines in the postnatal enamel of Individual C. An important finding of this part of the study is that childhood inoculations often – if not always – may leave a mark in enamel. This fact is extremely useful and even if the medical histories in these three case studies are not always 100% -accurate, the basis of a carefully controlled study now exists. If comprehensive written clinical histories can be released with ethical permission, these can be used to confirm or dispute the rates of enamel formation using the 'labels' at known ages which have been created by immunisation/vaccination/'jabs'. This fact may also explain similar lines that have often been noted in permanent enamel (within the cuspal enamel of first permanent molars) which can now be explained and made use of in new ways.

A further important finding is that events recorded in a medical history may not be the only events that leave accentuated markings in enamel. Some events that parents or clinical observers may think are significant may leave no markings, but others they don't recognise or ignore may actually cause disruption to developing enamel. Another thing to bear in mind is that the enamel maturation process may change or disguise an event, for example, the neonatal line which may be hypomineralised at birth, may during enamel maturation become equally as mineralised as the surrounding enamel; with only the original crystallite size and orientation remaining to identify the event in polarised light.

CHAPTER 8: Conclusions and Discussion

Summary discussions have been presented following each of the results sections and the issues discussed there will not be covered again in detail. The aim of the following is to discuss specific points related to crown formation times and the neonatal line and to present several suggestions for further work in this area.

8.1 Crown Formation Times

- **Daily enamel apposition rates established for each deciduous tooth type.**

Daily enamel apposition rates were established for each tooth type in this study. The total range for the daily rate of enamel apposition was from an absolute minimum of 2.07µm at the EDJ to an absolute maximum of 4.97µm at the enamel surface. These extreme rates vary less than those rates presented by Schour and Massler (1937), whose range for deciduous teeth was reported as 4-8µm. However Schour and Poncher (1937) refined this range in cervical enamel in the second molar to 3.6 to 4.3µm, with the total average being 3.92µm per twenty-four hours. This data compares with the equivalent area in this current study producing an extreme range of 2.08 to 3.20µm and a regional mean of 2.66µm per twenty-four hours.

Mean values of daily enamel apposition for both aspects for each region and each tooth type are shown in **Appendix Three**; the weighted averages calculated from this original raw data are shown in **Appendix Four**. The weighted mean for the rate of daily enamel apposition over both aspects, all three regions and for all tooth types is 3.23µm per twenty-four hours. As discussed earlier Schour and colleagues presented several papers stating the daily rate for deciduous enamel as being 4µm, which although is more comparable to the results obtained in this study than the 4-8µm range that was originally proposed, it is still high, however as reported in the introductory section of this thesis regarding this work, these rates are questionable.

In 1984, using geometry and statistics, Shellis calculated the daily rates of enamel formation for inner and outer enamel for deciduous teeth. These ranged from 4.5µm for the inner enamel to 5.3µm for the outer enamel for all deciduous tooth types. Beynon et al. (1998) using incremental analysis rather than 'tooth ring analysis' as used by Schour and colleagues, established the daily rate for deciduous enamel in the cuspal region of the second molar to range from 3.5µm in the inner enamel to 6.5µm in the outer enamel. More recently Mahoney (2011), using an archaeological sample, established the daily rate for deciduous enamel in the cuspal region of first molars to range from 2.9µm in the inner enamel to 4.9µm in the outer enamel, the equivalent minimum and maximum rates for the occlusal enamel of the first molar compared by Mahoney was 3.0µm to 4.4µm (Birch and Dean 2009), however from this current study using a larger sample size (eight rather than just one) this minimum to maximum range has now been refined to 2.51 to 5.07µm. For the second molar this was reported by Mahoney (2011) to range from 3.5µm in the inner enamel to 5.1µm in the outer enamel, the equivalent minimum and maximum rates for the occlusal enamel of the second molar compared by Mahoney was 2.2µm (incorrectly cited as 2.3µm) at the EDJ and 3.9µm in the outer occlusal enamel (Birch and Dean 2009). Mahoney also directly compared his results with those of the human second molar previously presented by Macchiarelli et al. (2006), however these results are for lateral enamel rather than occlusal enamel, this range is 2.3µm to 4.5µm. However from this current study using a larger sample size (eight rather than just one) this minimum to maximum range for occlusal enamel has now been refined to 2.07 to 4.63µm, see **Table 8.1**.

Table 8.1: This table shows a summary of the comparison of the recently published enamel formation rates in cuspal enamel of first and second molars.

Author	First Molar		Second Molar	
	Inner Enamel (minimum)	**Outer Enamel (maximum)**	**Inner Enamel (minimum)**	**Outer Enamel (maximum)**
Beynon et al. (1998)	-	-	3.5µm	6.5µm
Macchiarelli et al. (2006)	-	-	2.3µm*	4.5µm*
Birch and Dean (2009)	3.0µm	4.4µm	2.2µm	3.9µm
Mahoney (2011)	2.9µm	4.9µm	3.5µm	5.1µm
This study	**2.77µm**	**4.73µm**	**2.07µm**	**4.63µm**

*Lateral enamel.

Although the minimum values produced by this study are slightly earlier than those previously reported, these rates are much more comparable than those produced by 'tooth ring analysis' in the late 1930s and early 40s.

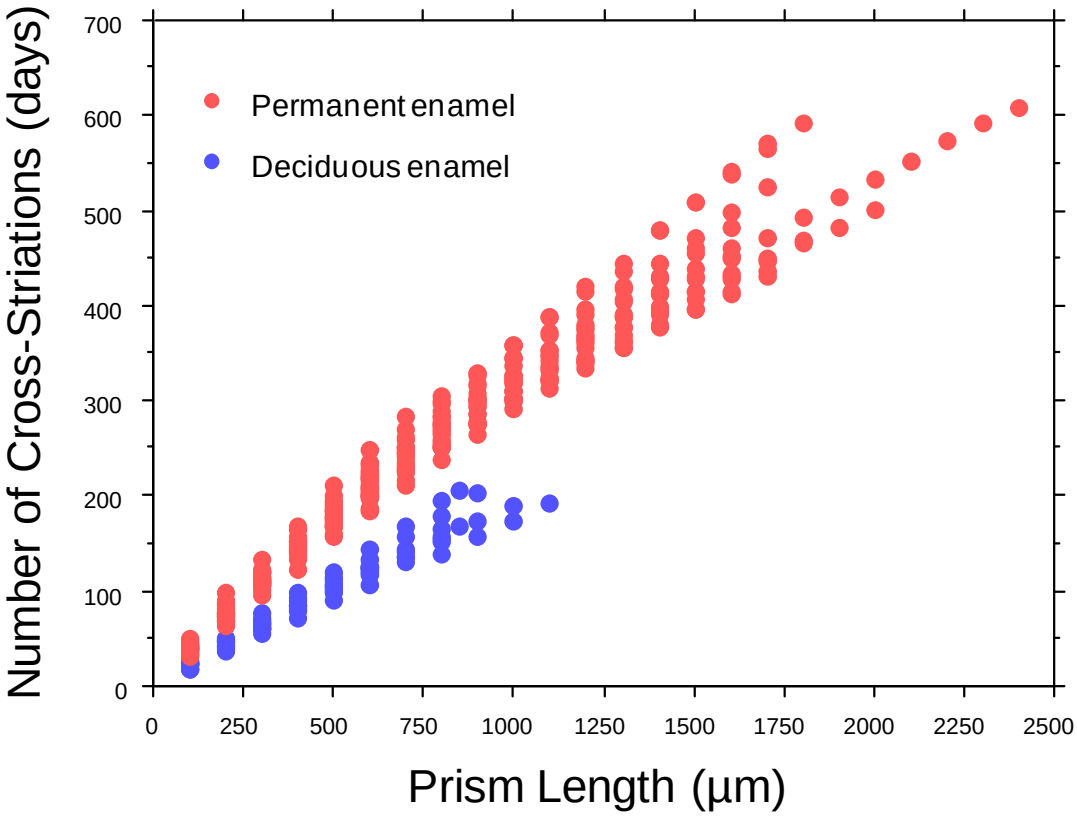
In 2009 FitzGerald and Hillson published their interim findings from their investigations of the excavations of the Ancient Greek cemetery site of Kylindra. This site uniquely consists of entirely infant burials. FitzGerald and Hillson (2009) presented the average daily enamel formation rate for incisors as being 3.7µm, which compares with the weighted averages from this study which range from 2.86µm in the cervical region to 3.14µm in the occlusal region, for canines this was reported as being 3.9µm, compared to 3.05µm in the cervical region to 3.29µm in the occlusal region and for first molars this was reported as 3.4µm compared to 3.44 to 3.71µm from this study. This mean rate of 3.4µm for the first molar also falls within the range of previously presented data (see **Table 8.1**). Although FitzGerald and Hillson do stress the fact that their sample consisted of premature births and infants who did not survive to full term, these daily rates of enamel formation are still more comparable than those originally suggested by Schour and Massler (1937).

Birch and Dean (2009) have previously established that rates of deciduous enamel formation are more consistent and do not increase over such a steep gradient as those known for permanent enamel formation and which have been reported as ranging from between 2.5 to 6.5µm per day (Dean 1998) and more recently from between 2.97 to 5.45µm per day (Mahoney 2008). Nevertheless, when the slowest forming deciduous enamel close to the EDJ in the cervical region is compared with the fastest forming enamel in the outer cuspal occlusal region (especially in the thicker enamel of the molars) the rates occlusally can be close to double those at the inner cervical region. Thus two parallel accentuated striae running from the lower cervix to the outer most cuspal occlusal enamel may diverge to almost twice their distance apart.

The fact that permanent crowns take longer to form than deciduous crowns was stated by Schour and Massler (1940a:1925) and attributed to the larger crown and '*slower rate of formation*'. Permanent occlusal enamel forms at a slower rate for a longer period of time commencing at the EDJ at a rate of about ~2.5-

3.0µm per day, but then it eventually rises to rates that come close to 6µm per day in outer occlusal regions (Dean 1998; Mahoney 2008). Shellis (1984:702) suggested that enamel formation rates were '*on average about five times greater in deciduous than in permanent teeth*'. These differences in the pattern of deciduous and permanent enamel formation rates underlie the differences observed between the linear nature of deciduous enamel formation and the non-linear trajectory of permanent enamel formation. This is reflected in the regression plot for 20 permanent occlusal trajectories and ten deciduous occlusal trajectories shown below and included here for comparison (**Figure 8.1**); the relevant regression formulae have also been included. It becomes clear, therefore, from this study that it is not possible to predict deciduous enamel formation times from equivalent data derived from permanent enamel.

Figure 8.1: This figure compares the cuspal enamel trajectories of ten deciduous teeth and twenty permanent teeth. The cumulative rate of enamel formation is greater in the deciduous enamel than it is in the permanent enamel. Adapted from Dean (2004).

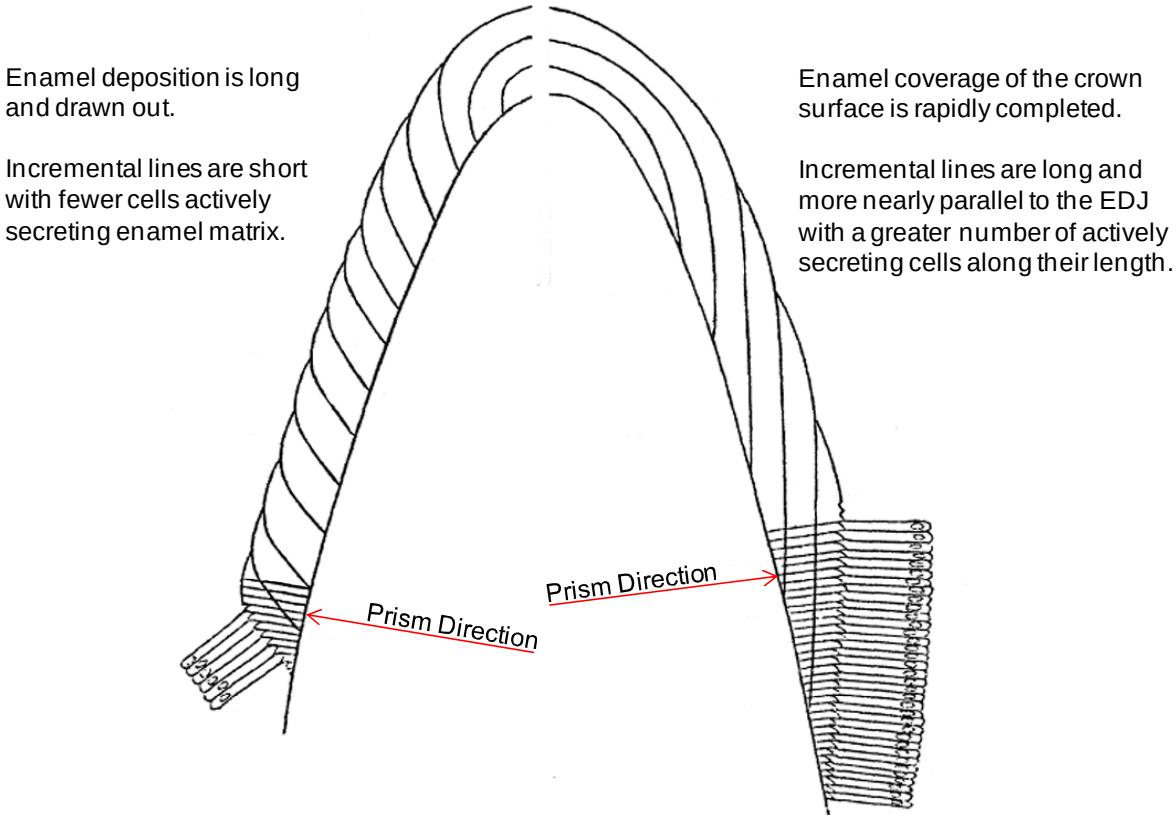


Permanent enamel: $Y = 8.669 + 0.367 * X - .000005216 * X^2$

Deciduous enamel:
Central Incisors: $Y = 0.248 * X + 6.731$
Lateral Incisors: $Y = 0.265 * X + 5.342$
Canines: $Y = 0.253 * X + 7.106$
First Molars: $Y = 0.254 * X + 3.291$
Second Molars: $Y = 0.274 * X + 11.548$

One general observation that should be discussed here is the appearance of the striae of Retzius (which although being very rare in the deciduous teeth are occasionally seen in the cervical enamel of the canine and second molar more clearly than elsewhere). Both these and any accentuated lines – and indeed the neonatal line itself – are more obliquely orientated to the EDJ than those striae in the permanent enamel. This observation was first published by Boyde (1964) who drew attention to the fact that oblique incremental lines result from many more ameloblasts being active at any one time (see **Figure 8.2**). This is the main reason why deciduous enamel crowns are able to form faster than permanent enamel crowns and it should be emphasized that the daily rate of secretion of enamel matrix is not the primary reason for differences in deciduous and permanent crown formation times. Tooth crown height and striae obliquity account for most of the faster deciduous crown formation times. In the cuspal region there is little difference between permanent and deciduous enamel striae orientation but more cervically, there is a more pronounced difference in the orientation of striae and accentuated lines to the EDJ.

Figure 8.2: This figure illustrates the difference in orientation of the enamel striae in permanent (on the left) and deciduous enamel (on the right). Adapted from Boyde (1964:Figure 6.3).



- **Increasing gradient of enamel formation rate from cervix to occlusal surface confirmed. Increasing gradient of enamel formation rate from EDJ to enamel surface established for deciduous enamel.**

Three developmental gradients have been described in the literature, these being:

1) That the daily enamel formation rate increases from the cervix to the occlusal surface (Massler and Schour 1946; Schour and Massler 1937; Schour and Poncher 1937). Although Kraus (1959a) and Kraus and Jordan (1965) also stated that teeth do not mineralise at the same rate vertically, they presented no further information regarding this.

   This gradient was confirmed by this study, the total weighted mean for the rate of daily enamel apposition over both aspects and all tooth types in the cervical enamel was 3.01µm and in the occlusal enamel this was 3.31µm, which although it is slight, does indicate the presence of an increasing cervical-cuspal gradient (Birch and Dean 2009).

2) That the daily enamel formation rate decreases from the anterior to posterior teeth (Schour and Massler 1937). Kraus (1959a) and Kraus and Jordan (1965) also stated that the maxillary central incisor appears to mineralise faster than the other teeth both vertically and mesio-distally, again unfortunately no further details are presented regarding this. From his observations using geometry, Shellis (1984:700) suggested that the '*average extension rate tended to decrease with increasing tooth size*'.

   The total weighted mean values in **Appendix Four** do seem to indicate that a gradient exists up to the first molars. However this is in direct opposition to that stated by Schour and Massler (1937). Starting with the central incisors the total weighted mean for each tooth type were 2.96µm, 3.05µm, 3.21µm and 3.58µm for the first molar, however for the second molar the rate was 3.11µm. So if anything the rate of formation seems to increase from anterior to posterior rather than the other way round.

3) That the daily enamel formation rate reported as following the '*law of gradients*' by Schour and Massler (1940a:1921) is incorrect. Schour and Massler (1940a:1918) stated that this law of gradients is illustrated by the fact that '*cellular activity begins at maximal velocity*' and that increments nearest to the growth centre are farther apart than those increments nearer the enamel surface. Massler and Schour stated that this decrease in rate is due to the '*increase of age of the formative cell (age gradient)*' (1946:147). This is in direct contradiction to what was found in the current study.

   As in point 2 above, this point also contradicts what was found in this study and in this case it is the exact opposite (Birch and Dean 2009). The mean value of enamel formation increases in all tooth types from the EDJ to the enamel surface, for example the mean in the second molar increases in the cervical enamel from 2.49µm at the EDJ to 2.79µm at the enamel surface, from 2.55 to 4.00µm in the lateral enamel and from 2.48 to 3.74µm in the occlusal enamel (see **Appendix Four**).

This increase in the rate of enamel formation was in fact originally suggested in 1927 by Mellanby, but was regarded as '*not true*' by Massler et al. (1941:62). Shellis (1984) identified a mean increase in the enamel formation rate from inner to outer enamel of 20% and more recently Mahoney (2011) has also confirmed that rates of formation increase in the cuspal enamel from the EDJ to the enamel surface in both first and second molars.

So out of the three gradients previously described in the literature one was definitely confirmed (1) and two were definitely refuted (2 and 3).

- **Regression formulae developed for each tooth type to allow calculation of crown formation times without having to count daily cross-striations.**

The data presented in this study are derived from a comparatively small sample of human deciduous teeth. Nonetheless, the results are consistent enough to suggest that it is possible to use these data to estimate the crown formation times of deciduous teeth from other ground sections at defined confidence limits. This in effect enables longitudinal growth data to be retrieved from the

incremental structure of enamel and it also permits the estimation of the chronological age for juvenile human remains from developing enamel crowns.

Regression formulae were developed for each tooth type to allow the calculation of crown formation times without having to count daily cross-striations. It can be seen from **Table 6.3** that on average 100µm of enamel at the EDJ form at an average rate of 3.12µm per day (100µm/mean number of days = 32 days) whereas at the outer enamel at 800µm in the thicker enamelled deciduous second molars this average is closer to 3.46µm per day (800µm/mean number of days = 231 days). The estimates made here from the regression equations are, however, in effect 'smoothed out' and many local fluctuations in the enamel formation rate in an individual tooth are not taken account of. Notably, the decrease in the formation rate of approximately 0.5µm per day immediately after the neonatal line (Birch and Dean 2009), or any other accentuated marking that may be specific to an individual tooth are not accounted for by these regression formulae. It follows that the formation times they generate remain 'estimates' and will always be less reliable than when every daily increment in a tooth section is counted directly.

The use of the regression formulae in **Chapter 7** allowed a direct comparison between the daily cross-striation counts and the results produced by the formulae. In the closest match of these three cases (Twin B) these daily cross-striation counts and the results produced by the formulae corresponded exactly on eight out of 28 (28.5%) occasions. In the worst case (Individual C) there were only two direct comparisons out of 11 (18%). However, unlike the other two individuals the cross-striation counts in Individual C fell outside of the 95% confidence limit on four occasions, with the discrepancy on these occasions ranging from a minimum of five to a maximum of 20 days. Interestingly on all four of these occasions the cross-striations were particularly difficult to count. In **Chapter 7** a direct comparison was also made between the results produced by the formulae and the medical histories of the three juveniles. Again achieving a very close time-match between the formulae results and the actual histories. This demonstrates how the use of the formulae can overcome the problem of areas of enamel where cross-striations are not clearly visible. The use of such formulae also saved a considerable amount time in the estimation of crown

formation times, which is particularly useful in forensic work when the police require a speedy response as to the age of an individual. However, one limitation to the use of the regression formulae to estimate postnatal crown formation in order to help determine the age of an individual in forensic cases is that the formulae can only be applied to teeth that are still forming, as once the enamel is fully formed and the root begins to form, the biological enamel clock has ‘stopped’ and this method will no longer give the age of the individual and instead will age the formation time of the crown. The findings of this study can be used to contribute to age at death estimates for infants aged up to 12 months.

Good incremental markings in deciduous enamel are often hard to find and the process is quite time consuming, however the approach outlined in this study makes estimates possible by utilizing the regression formulae that are presented. The prerequisites being that the direction of the enamel prisms is visible and there are sufficient accentuated markings or long-period striae visible in order to track enamel formation from the region of the dentine horn to the cervix.

As long as the prism length measured is under the maximum distance used in the formulae, this works – it could not be used for the second molars in the case of the twins and Individual C as the length along an enamel prism measured from the EDJ to some accentuated lines exceeded that of any of the individuals used to generate the formulae in the first place. It must be remembered that the regression equations can only be used for prism lengths that are below those used to develop the regression equations.

In future, larger sample sizes will undoubtedly both improve the accuracy with which this can be done and also explore the possibility that differences in enamel formation rates may exist, for example, between sex, geographical regions worldwide and/or between individuals of different socioeconomic backgrounds or between modern and archaeological populations.

As result of the development of the regression formulae the following now become possible:

- Prenatal enamel crown formation times established for each tooth type.
- Deciduous enamel initial crown mineralisation times established for each tooth type.
- Sequence of initial mineralisation established for deciduous dentition.
- Postnatal enamel crown formation times established for each tooth type.
- Proportion of enamel present at birth established for each tooth type.
- Sequence of crown completion established for deciduous dentition.
- Deciduous enamel total crown completion times established for each tooth type.

- **Prenatal enamel formation times established for each tooth type.**

It was only after the identification and confirmation that the neonatal line did in fact have a neonatal origin that it became possible to accurately determine the difference between pre- and postnatal enamel. This biological landmark allowed researchers to separate these two types of enamel more precisely than had been done previously. This breakthrough also meant that it was now possible to establish the time of initial mineralisation for deciduous enamel in utero, without the requirement of fetal specimens and this removed the major problem of the accurate aging of the specimens. Using the neonatal line as a biological landmark, the regression equations were applied to determine the amount of time taken to form the prenatal enamel for each tooth type. The time taken to develop the prenatal enamel of the crowns of the deciduous dentition ranged from 16.8 weeks for the second molar to 20.6 weeks for the central incisor (see **Table 6.4**).

One limitation of estimating the period of prenatal enamel formation that leads to inaccuracy is section obliquity. Very small shifts in the plane of section have large effects on measurements of the true linear distance between the tip of the dentine horn and the neonatal line. This is especially so in the case of deciduous teeth where such small and often pointed cusps make it hard to produce ground sections in the true plane of section.

- **Deciduous enamel initial crown mineralisation times established for each tooth type.**

Using the times established for prenatal enamel formation, it was then possible to ascertain the crown initiation times for each tooth type by calculating backwards from birth. This was done by subtracting the mean number of days of prenatal enamel formation from the duration of an average pregnancy which is 39 weeks for an average singleton birth (Davidoff et al. 2006). The mean average and the 95% confidence limits range for initial mineralisation are shown in **Table 8.2**, along with the data collated from the literature in order to allow a direct comparison. The reader is again reminded of the possible additional two week range due to aging of the fetal specimens in the data presented (see **Section 3.6.1**).

When compared to the data collated earlier in this work detailing crown initiation times (**Table 8.2**), not surprisingly the initiation times obtained using the regression formulae fall within the range of those in this table, the reason that this is not surprising is due to the fact that the range in this data is so large. The limiting factors such as methods of observation have been dealt with in **Chapter 3**. However, when compared to the results obtained using 'tooth ring analysis' (see **Table 3.5**) which is the nearest comparable method of study (Kronfeld and Schour 1939; Schour and Kronfeld 1938; Schour and Massler 1940a) the results are somewhat similar. Except for the lateral incisor (which is one week later than that obtained by 'tooth ring analysis') and the second molar (which is one week earlier than that obtained by 'tooth ring analysis'), the results obtained by 'tooth ring analysis' fall within the range established by this study. What is interesting is that the time of 22 weeks which was obtained using the regression formulae, corresponds with the 1938 study but not with the 1939 study, where there is a discrepancy of one week, unfortunately no explanation of why the original time was increased to 24 weeks in 1939 is given (Kronfeld and Schour 1939).

Table 8.2: Initial mineralisation table showing data collated from the literature review in chronological order, expressed in gestational weeks. If required conversion to weeks was performed, original data is in parentheses. Where information was available mandibular and maxillary teeth are presented separately.

Author	Date Of Publication	Form Of Original Data	Type Of Conversion	Central Incisor		Lateral Incisor		Canine		First Molar		Second Molar	
				Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular
Robin & Magitot	1860-63	Days	Days / 7	-	11.43-12.14 (80-85 days)	-	13.43-14.14 (94-99 days)	-	17.14-17.86 (120-125 days)	-	12.43-13.14 (87-92 days)	-	15.43-16.28 (108-114 days)
Peirce	1877	Weeks	None Required	17		17		17		18		18	
Legros & Magitot	1880	Weeks	None Required	16	16	16	16	16 in text. 17 in table.	16	17	17	17	17
Peirce	1884	Weeks	None Required	17		17		17		18 in chart. 19 in text.		18 in chart. 19 in text.	
Tomes	1889	Weeks	None Required	17		17		17		18		18	
Broomell & Fischelis	1913	Months	Months x 4	16 (4 mths)	16 (4 mths)	16 (4 mths)	16 (4 mths)	20 (5 mths)	20 (5 mths)	20 (5 mths)	20 (5 mths)	20-24 (5-6 mths)	20-24 (5-6 mths)
Tomes	1914	Weeks	None Required	20		20		24		24		24	
Mummery	1924	Weeks	None Required	20		20		24		24		24	
Brady	1924	Weeks	None Required	17		17		17		20		20	
Churchill	1932	Months	Months x 4	18 (4.5 mths)		18 (4.5 mths)		22 (5.5 mths)		20 (5 mths)		22 (5.5 mths)	
Wolfe	1935	Weeks	None Required	17 in text. 20 in chart.		17 in text. 20 in chart.		24		24		24	
Meyer	1935	Months	Months x 4	20 (5 mths)		20 (5 mths)		24 (6 mths)		20 (5 mths)		32 (8 mths)	
Kronfeld	1935c & 1937	Months	Months x 4	20 (5 mths)		20 (5 mths)		24 (6 mths)		20 (5 mths)		24 (6 mths)	
Schour & Kronfeld	1938	Months	Months x 4	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		22 (5.5 mths)	
Kronfeld & Schour	1939	Months	Months x 4	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		24 (6 mths)	
Schour & Massler	1940a	Months	Months x 4	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		24 (6 mths)	
Kraus	1959b	Weeks	None Required	12-16		-		-		-		14-22	
Turner	1963	Weeks	None Required	-		-		-		18		19-20	
Nomata	1964	Weeks	None Required	17	17.66 (17⅔)	19.66 (19⅔)	17.66 (17⅔)	21.33 (21⅓)	19.66 (19⅔)	19.33 (19⅓)	19.66 (19⅔)	21.33 (21⅓)	23.66 (23⅔)
Kraus & Jordan	1965	Weeks	None Required	14		16		17		15.5		19	18
Lunt & Law (average)	1974	Weeks	None Required	14		16		17		15.5 (15½)		19	18
Lunt & Law (range)	1974	Weeks	None Required	13-16		14.66-16.5 (14⅔-16½)	14.66 (14⅔)-	15-18	16-	14.5 (14½)-17		16-23.5 (23½)	17-19.5 (19½)
Sunderland et al.	1987	Weeks	None Required	15-19		16-21		19-22		16-19		20-22	
Mahoney	2011	Days	Days / 7	-		-		-		-	19-26	-	25-31
Birch (average)*	**2011**	**Days**	**Mean days / 7**	**-**	**18**	**-**	**20**	**-**	**21**	**-**	**19**	**-**	**22**
Birch (range)*	**2011**	**Days**	**Mean days / 7**	**-**	**17-19**	**-**	**19-20**	**-**	**20-22**	**-**	**18-20**	**-**	**22-23**

*** 273 days (39 weeks) - mean CFT before birth (days) /7**
*** 273 days (39 weeks) - 95% confidence limits (days) /7**

Recent work by Mahoney (2011), who was investigating the incremental structure of the mandibular molars, presented mean prenatal formation times of 113 days for first molars and 74 days for second molars. When compared to the times obtained from this study for the first molars there is a discrepancy of 27 days from the mean of 140 days and for the second molar there is a discrepancy of 44 days, from the mean of 118 days (see **Table 6.4**). Mahoney also presented a range of 49 days for the first molar and of 42 days for the second molar (see **Table 8.2**), which when compared to the range in this study, of 11 days for the first and 8 days for the second molar, seems extensive (see **Table 6.4**).

- **Sequence of initial mineralisation established for deciduous dentition.**

The sequence of initial mineralisation established by this study commences with central incisor, first molar, lateral incisor, canine and then second molar. When compared to the method using 'tooth ring analysis' these results do not correspond, as Schour and Massler (1940a) reported this sequence commences with the anterior teeth and progressing posteriorly (see **Table 3.8**). However, as discussed earlier (**Section 3.5.2**) the sequence proposed by Kraus and Jordan (1965) and supported by Lunt and Law (1974) is also the one established by this study.

- **Postnatal enamel formation times established for each tooth type.**

As with the prenatal enamel formation times it was only after the identification and confirmation that the neonatal line did in fact have a neonatal origin that it became possible to accurately determine the difference between pre- and postnatal enamel. Using the neonatal line as a biological landmark, the regression equations were applied to determine the amount of time taken to form the postnatal enamel for each tooth type. The time taken to develop the postnatal enamel of the crowns of the deciduous dentition ranged from 13.7 weeks for the central incisor 55.5 weeks for the second molar (see **Table 6.4**).

These results are presented in **Table 8.3** along with the data obtained from the literature review in order to allow a direct comparison. When compared to the

data collated earlier in this work detailing crown completion times, the times obtained using the regression formulae unlike the initiation times, are very different from those reported previously. The average results obtained in this study increase the time of crown formation from a minimum of three weeks to a maximum of 15 weeks from the previously recorded times obtained by 'tooth ring analysis'. The greatest discrepancy being the second molars which Kronfeld and Schour (1939) and Schour and Massler (1940a) stated completes at 40 weeks, however the results obtained in this study increase this completion time to 53-58 weeks.

It is possible that the use of the regression formulae developed for deciduous teeth does not work as well on the second molars, since the application of the formula failed in the attempt to establish times for accentuated striae in the three case studies. This might have been due to the fact that the distances being measured were quite small compared to the initial numbers in the formulae. However, the results obtained here suggest the second molar may form more like a permanent tooth than a deciduous tooth. The growth of the second molar was identified as being different from that of the other deciduous teeth and this is illustrated in **Figure 6.1b** with the second molar forming at a much slower rate that the other teeth. Unfortunately it appears that at the moment the regression formulae should only be applied to second molars to estimate crown formation times with some caution until more substantial data become available to increase the reliability of the regression formulae.

However, recent work by Mahoney (2011), who was investigating the incremental structure of the mandibular molars presented postnatal formation times of 275 days for first molars and 396 days for second molars. When compared to the times obtained from this study (see **Table 6.4**) for the first molars there is a discrepancy of 89 days from the mean of 186 days, unfortunately Mahoney does not present any ranges with this data, so it cannot be determined whether the results established in this study fall within his range. However, for the second molar there is a discrepancy of only seven days, between these two studies from the mean of 389 days.

- **Proportion of enamel present at birth established for each tooth type.**

The amount of enamel present at birth for each tooth type was difficult to compare with those amounts determined by previous authors; this is due to the fact that many of the historical studies present descriptive text for the amount of enamel formed for each tooth type at birth. Where it has been possible to convert the data obtained in the past to percentages the amounts appear to be similar. The results obtained from this study are shown in **Table 8.4**, along with the data obtained from the literature in order to allow a direct comparison. When compared directly with the limited percentages available from 'tooth ring analysis' the largest discrepancy (6%) is in the lateral incisor.

- **Sequence of crown completion established for deciduous dentition.**

The sequence of crown completion established by this study commences with the central incisor, lateral incisor, first molar, canine and then ends with the second molar. When compared to the methods using 'tooth ring analysis' these results correspond with those obtained by both Kronfeld and Schour (1939) and Schour and Massler (1940a).

- **Deciduous enamel total crown completion times established for each tooth type.**

By the addition of the times taken for prenatal and postnatal enamel formation it, was then possible to establish the total crown completion times for each tooth type. The results obtained are shown in **Table 6.4**.

Schour and Massler (1940a:1925) stated that the canine '*consumes the longest time because of the length of its crown*', however this is not what was found in this study, the longest forming tooth was the second molar which took a total of 16.64 months to complete. Mahoney (2011), established that the total crown formation times for the first mandibular molar was 388 days and for the second was 470 days, the discrepancy between the study by Mahoney and this study for the first molar is 62 days from the mean of 326 days and for the second molar is 36 days from the mean of 506 days.

Table 8.3: Crown completion table showing data collated from the literature review in chronological order expressed in weeks after birth. Conversion to weeks was performed, original data is in parentheses. Where information was available mandibular and maxillary teeth are presented separately.

Author	Date Of Publication	Form Of Original	Type Of Conversion	Central Incisor		Lateral Incisor		Canine		First Molar		Second Molar	
				Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular
Broomell & Fischelis	1913	Months	Months x 4	8 (2 mths)		8 (2 mths)		8 (2 mths)		8 (2 mths)		8 (2 mths)	
Meyer	1935	Months	Months x 4	12 (3 mths)		12 (3 mths)		48 (1 year)		36 (9 mths)		-	
Kronfeld	1935c & 1937	Months	Months x 4	16 (4 mths)		20 (5 mths)		36 (9 mths)		24 (6 mths)		40-48 (10-12 mths)	
Kronfeld & Schour	1939	Months	Months x 4	6 (1.5 mths)	10 (2.5 mths)	10 (2.5 mths)	12 (3 mths)	36 (9 mths)	36 (9 mths)	24 (6 mths)	22 (5.5 mths)	44 (11 mths)	40 (10 mths)
Schour & Massler	1940a	Months	Months x 4	6 (1.5 mths)	10 (2.5 mths)	10 (2.5 mths)	12 (3 mths)	36 (9 mths)	36 (9 mths)	24 (6 mths)	22 (5.5 mths)	44 (11 mths)	40 (10 mths)
Lunt & Law	1974	Months	Months x 4	6 (1.5 mths)	10 (2.5 mths)	10 (2.5 mths)	12 (3 mths)	36 (9 mths)	36 (9 mths)	24 (6 mths)	22 (5.5 mths)	44 (11 mths)	40 (10 mths)
Mahoney	2011	Days	Days / 7	-		-		-		-	39	-	56
Birch (average)	**2011**	**Days**	**Mean days / 7**	**-**	**13**	**-**	**16**	**-**	**43**	**-**	**26**	**-**	**55**
Birch (range)	**2011**	**Days**	**Mean days / 7**	**-**	**12-15**	**-**	**15-17**	**-**	**40-46**	**-**	**25-28**	**-**	**53-58**

Table 8.4: Proportion of crown completed at birth. Derived from data collated from the literature review in chronological order expressed as percentage of completion, where required conversion to percentage was performed, original data is in parenthesis. Where information was available mandibular and maxillary teeth are presented separately.

Author	Date Of Publication	Central Incisor		Lateral Incisor		Canine		First Molar		Second Molar	
		Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular	Maxilary	Mandibular
Peirce	1884	'quite complete'		'quite complete'		66.66% (2/3)		66.66% (2/3)		50% (1/2)	
Mummery	1924	'crowns calcified'		'crowns calcified'		'tips calcified'		'cusps united'		'cusps united'	
Churchill	1932	50% (1/2)		40% (2/5)		25% (1/4)		'cusps united'		20% (1/5)	
Hess et al.	1932	66.66% (2/3)		66.66% (2/3)		25% (1/4)		'little more than the occlusal surface'		'base of the cusps' 'incompletely calcified	
Meyer	1935	'almost complete'		'half complete'		-		-		'crown tips have coalesced'	
Kronfeld & Schour	1939	83.33% (5/6)	60% (3/5)	66.66% (2/3)	60% (3/5)	33.33% (1/3)		'cusps united'		'cusp tips still isolated'	
Schour & Massler	1940a	83.33% (5/6)	60% (3/5)	66.66% (2/3)	60% (3/5)	33.33% (1/3)		'cusps united'		'cusp tips still isolated'	
Lunt & Law	1974	83.33% (5/6)	60% (3/5)	66.66% (2/3)	60% (3/5)	33.33% (1/3)		'cusps united; occlusal completely calcified plus half to three fourths crown height'	'cusps united; occlusal completely calcified'	'cusps united; occlusal incompletely calcified; calcified tissue covers a fifth to a fouth crown height'	'cusps united; occlusal incompletely calcified'
Mahoney	2011	-		-		-		-	29%	-	19%
Birch	**2011**	**-**	**59.99%**	**-**	**54.81%**	**-**	**29.87%**	**-**	**43.01%**	**-**	**23.23%**

8.2 Neonatal Line

- **Identification of the importance of the position of the neonatal line.**

As discussed in **Chapter 4** many authors have studied the nature and position of the neonatal line in modern human deciduous teeth (see **Section 4.3**). The use of the regression formulae developed here allows the position of the neonatal line in the enamel crown to be quantitatively established and for its time of formation to be determined at defined confidence limits. In relatively unworn deciduous teeth, it is possible to measure the prism length from the dentine horn to the neonatal line along a prism path and then using the regression formulae presented here, the time of formation can be determined. It is then possible to identify whether or not birth occurred at full term. This was demonstrated in the case study of the twins, (see **Section 7.2.1**) when the position of the neonatal line was found to be nearer to the EDJ than expected for both twins. When the prenatal enamel formation times were compared to those obtained in this study, it was suggested that the twins had been born 62 days prematurely; on comparison with the medical history it was found that this predicted time of 62 days was inaccurate by only six days.

It is relatively easy to apportion the prenatal and postnatal periods of enamel formation within the whole time taken to form a crown and if records exist, to compare these in cases of children born either prematurely or late. Although more work is required using the regression formulae to determine whether a birth is premature or not, this is a very promising start.

- **Identification of decrease in enamel formation rate after the neonatal line and subsequent recovery phase.**

This study together with Macchiarelli et al. (2006) and Birch and Dean (2009) are the first methodical studies to demonstrate a hypoplastic reaction in deciduous enamel subsequent to birth, which is then followed by a recovery phase immediately afterwards. Rates of enamel formation immediately after the neonatal line often decrease by an average of 0.5µm per day but then start to recover within a 100µm (roughly one month) zone. This recovery lasts for a

maximum of approximately 400µm from the neonatal line before the formation rate completely returns to its original growth trajectory.

Reduced rates in enamel formation following the occurrence of the neonatal line in humans have also recently been confirmed by Mahoney (2011). It is possible that these reduced rates were also observed by Beynon et al. in their comparison of human and Proconsul teeth in 1998, however the link between this decrease in enamel formation and the occurrence and position of the neonatal line was not noticed at this time (Dean pers. com. August 2011). Beynon et al. (1998:Figure 8) have included a box plot for a human second deciduous molar which is very similar to the results obtained in this study. In this second molar there is a decrease in the enamel formation rate of about 0.3µm which occurs in the third month. This compares well to the mean amount of prenatal enamel in the second molar derived using the regression formulae and which takes 3.86 months to form (see **Table 6.4**). Following this decrease in the formation rate, the rate then appears to recover during the following month, just as found in this study.

Despite the fact that teeth and brains are usually considered resilient to environmental stress, this study shows that deciduous enamel responds in the same way to stressful events just as other tissues do, for example, bone, cartilage, muscle and fat, (Harris 1933; Sontag 1938). It is well known that the physiological changes at birth are associated with loss of weight, autophagy, acidosis, etc. (Heintz 2004; Kuma et al. 2004; Okada 1943). Besides the physical appearance of the neonatal line in human deciduous teeth, it is now clear that enamel hypoplasia either associated with or without a line, is an equally good measure of stress during deciduous crown formation.

- **Identification of decrease in enamel formation rate after accentuated striae and subsequent recovery phase.**

Similar to the decreasing rates of enamel formation that were identified following the occurrence of the neonatal line, decreasing rates were also observed occurring after other accentuated striae of Retzius, these have been termed ‘stress lines’ in this study. The exact cause of these lines is as yet unknown, although they appear to be linked with systemic metabolic

disturbances; one recent suggestion has been that stress lines in the permanent enamel of baboons are caused by severe environmental and psychological stresses (Dirks et al. 2002).

Following such a 'stress line' the developing enamel seems to react in the same way it does following the occurrence of the neonatal line, in that a period of recovery can be seen to occur following the decrease in enamel formation after the event. A recovery or 'catch-up' period is commonly seen in other tissues of the body and it often occurs after periods of juvenile illness or starvation (Boersma and Wit 1997; Lee and Myers 1979; Osborne and Mendel 1916; Prader et al. 1963; Williams 1981)

Mahoney (2008:143) identified a '*sharp reduction and slow recovery*' of the enamel formation rate in two permanent first molar cusps from the same individual from an archaeological assemblage. Mahoney (2008:145) suggested that this tooth may have '*retained a record of a systemic event*' in response to a '*type of juvenile illness that corresponds to some types of hypoplasia*'. His data indicates that the decrease in formation occurred over 34 days in the hypoconid and 36 days in the entoconid, which is similar to the findings of this study (~30 days). The recovery time illustrated by Mahoney's data also suggests a comparable recovery period, being 105 days in the hypoconid and 109 days in the entoconid. In the deciduous teeth the maximum recovery period was approximately 400µm (roughly four months) from a 'stress-line' in the deciduous second molar (see **Figure 6.20: E2**). However, unlike the decreased rates of enamel formation reported in this study, Mahoney was unable to identify any corresponding accentuated 'stress-line'.

A similar reduction in the amount of enamel formation, (inferred from a reduction in spacing between Retzius lines) has also been shown to correspond to surface hypoplasia in the permanent teeth of wild boar and domestic pigs (Witzel et al. 2006). While recovery in enamel secretion after '*reduced enamel matrix formation*' following a systemic insult (fluoride induced disturbance), has also been reported for the permanent teeth of roe and red deer by Kierdorf and Kierdorf (1997:125).

So again it seems that besides the physical appearance of a neonatal line or a ‘stress line’ in human enamel, enamel hypoplasia either associated with or without such a line is an equally good measure of stress during deciduous crown formation, as well as in permanent crown formation (Mahoney 2008).

- **Comparison with medical records confirmed high levels of stress and illness observed as accentuated striae in enamel.**

Following on from the work of Rushton in 1933, when he attempted to compare ground sections with known medical histories the regression formulae were applied to the teeth of three individuals each with a known medical history.

For Twin A the regression formulae resulted in predicting the exact day of two recorded medical events out of six, on two other occasions the day of a medical event fell within the 95% confidence limits. For Twin B the regression formulae resulted in predicting the exact day of a recorded medical event on five out of 12 occasions, on three other occasions the day of a medical event fell within the 95% confidence limits. Unfortunately for Individual C the use of the regression formulae did not achieve any exact matches although on two out of six occasions the recorded medical event fell within the 95% confidence limits (the possible reason for this discrepancy is discussed in **Chapter 7**).

This comparison between ground sections and a known medical history generally appears to support the use of the regression formulae. However, a successful comparison will depend on the accuracy and the type of information recorded in the medical notes. It is the lack of a suitable medical history that appears to have been the reason why Rushton was unsuccessful. The type of information that he had to work with ranged from ‘*a bad burn at 6 months*’ to ‘*fell on head at nine months but all right the next day*’ (1933:170). Similar difficulties were also experienced by Suckling et al. (1987:1466) who in their investigation of developmental enamel defects, stated that ‘*few parents can remember the date, duration, and severity of all illnesses experienced in their children’s first five years of life*’.

However with a ‘good medical history’ as provided for the twins the use of the regression formulae appears to be successful, however, with a poor or almost non-existent medical history as in the one provided for Individual C, although the dates may not correspond exactly, the general trend may be indicative of the state of health of the individual. This was seen to be the case for Individual C where although there initially appeared to be no correspondence between the history and the ‘stress lines’ the general trend of the history could be matched although roughly, to possible medical events. However, although not ideal this may still be of use to the forensic osteologist and odontologist in trying to establish the identity of juvenile remains.

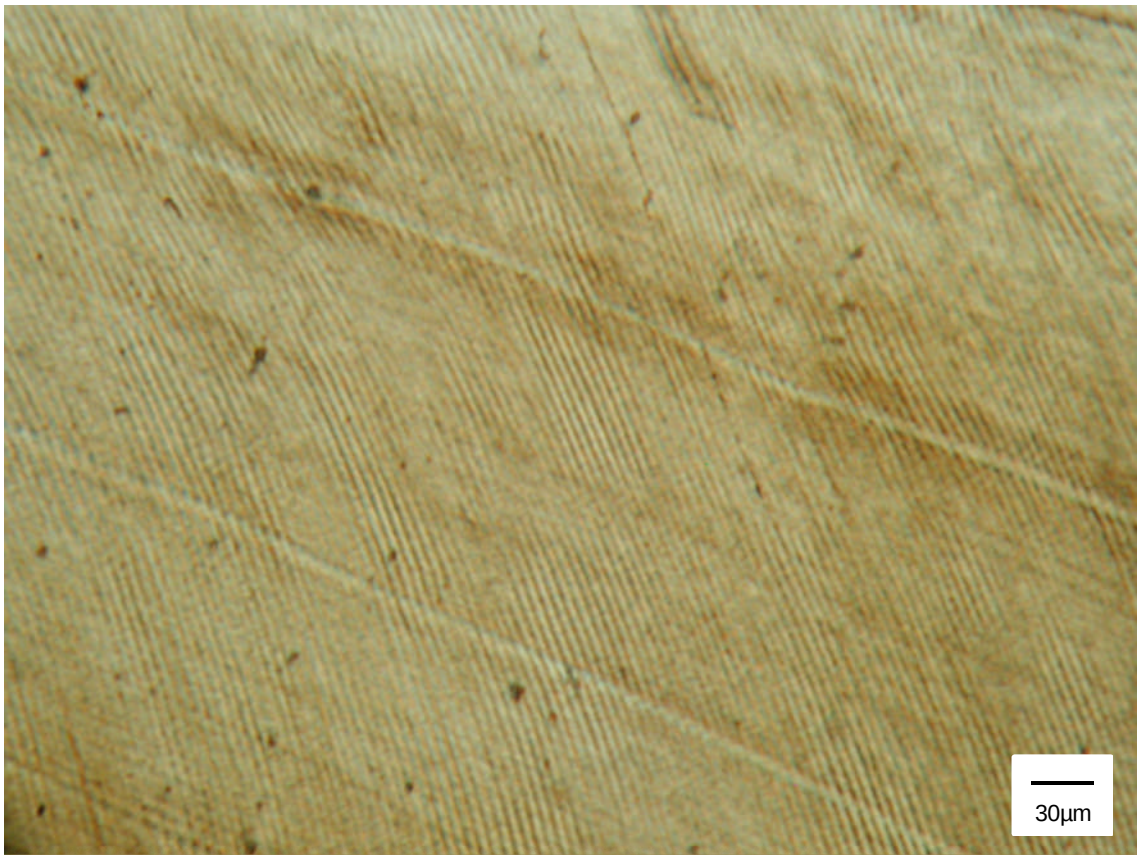
A further finding is that events recorded in a medical history may not be the only events that leave accentuated markings in enamel. Several accentuated lines were identified which did not correspond at all to anything on the medical histories. So it must be remembered that what is recorded by a clinician may not necessarily be analogous with a hypoplastic event. Likewise some events that parents or clinicians may think are significant may not leave any trace at all, for example those entries cited by Rushton above, while other events that parents or clinicians don’t recognise or ignore may actually cause disruption to developing enamel. Rushton (1933:171) stated that if the rate of enamel formation is more or less consistent, ‘*then one must conclude that, whatever kind of disturbance gives rise to these random lines, it is not necessarily memorable or much of an outward sign*’. Mahoney (2008:145) also added that ‘*clearly some enamel defects are more marked than others, even within the same tooth*’.

As mentioned in **Section 7.3**, another thing to bear in mind, is that one complication of interpreting accentuated lines is that during the enamel maturation phase many areas or patches of enamel that were originally hypomineralised, become mineralised to the same degree as the surrounding enamel. As a result the enamel maturation process may change or disguise an event, for example, the neonatal line which may be hypomineralised at birth may, during enamel maturation become equally as mineralised as the surrounding enamel, with only the original crystallite size and orientation remaining to identify the event in polarised light.

- **Existence of 'Vaccination/Immunisation lines' established.**

Another finding to emerge from the comparison of the ground sections with the known medical histories was the appearance of several accentuated striae with very similar appearances in all three individuals. These lines were prominent and bright and especially more so in the teeth from Individual C (see **Figure 8.3**). These lines corresponded with the occurrence of the DTP vaccinations. In the case of Individual C, even though the mother's oral history was clearly inaccurate with regard to the exact timing of specific medical events, it was clear that DPT vaccinations had been given at regular intervals.

Figure 8.3: This figure shows an example of two DTP 'vaccination lines'.

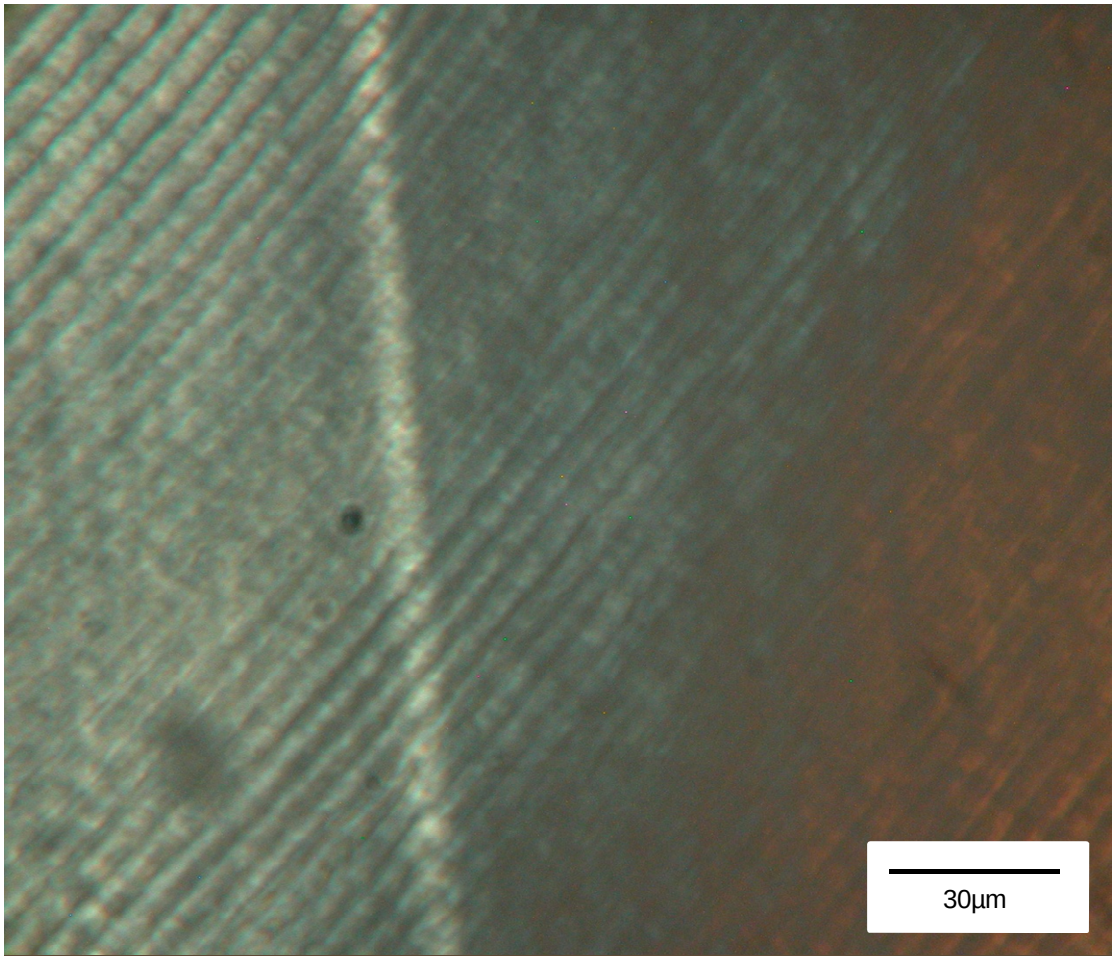


Schour and Poncher in 1937, had previously injected sodium fluoride into a child with inoperable hydrocephalus at known intervals, they then identified a corresponding accentuated stria for each of these injections. Although no further details are provided about this injection procedure it appears that they gave the patient '*one subcutaneous and twenty-four intravenous injections*' (1937:764). As they managed to successfully identify a maximum of 20 of the

25 injections it seems reasonable to suggest that these accentuated striae were the result of a foreign chemical being introduced into the system rather than stress induced from the injection process itself. This suggestion is supported by the work of Schour and Hoffman (1939b) who used intravenous and intraperitoneal injection techniques to inject alizarin red S (sodium sulphalizarate) into rhesus macaque monkeys; from their data it does not appear that the particular method of injection used influenced the results obtained. In addition, Okada (1943) used intravenous, intraperitoneal and subcutaneous injections in his work and again the resultant accentuated striae appear to be due to the introduction of a foreign chemical into the body rather than the injection technique used.

In rats, injections of sodium fluoride produced '*sharp effects in the form of bands of defective pigmentation and calcification in the enamel*' (Schour and Poncher 1937:758). The effects of sodium fluoride injections were recognised histologically in the ameloblasts of rats which were killed within one to 12 hours of a single sodium fluoride injection, (Schour and Hoffman 1939b; Schour and Smith 1934a; Schour and Smith 1934b). When they observed the result of the sodium fluoride injections in enamel Schour and Smith (1934b) and Schour and Hoffman (1939b) reported that it consisted of a pair of light (disturbed) and dark (recovery) incremental layers for each injection. They suggested that the light layers represented the immediate primary response to the injection and are '*imperfect in formation and calcification*', while the dark layers represented the secondary recovery response and are '*normal in formation and normal or excessive in calcification*' (Schour and Smith 1934b:2). Schour and Hoffman (1939b:166) also reported that sometimes a '*short bend*' is present in the course of the enamel prisms, which indicates a '*disturbance in the path of formation*'. This was not the case in this study as the prisms seem to pass straight onwards through the vaccination/immunisation line with no bending at all, which implies that these accentuated striae may be the result of a hypomineralisation rather than a hypoplastic event (see **Figure 8.4**). This is unlike the neonatal line which does appear to be the result of a hypoplastic event, as the prisms do deviate from their normal course and on occasions appear to break up within the neonatal line before continuing on into the postnatal enamel.

Figure 8.4: This figure shows the continuation of the prism path through a 'vaccination/immunisation line'.



The vaccination/immunisation lines that were identified in this study appear to be the result of a hypomineralisation rather than a hypoplastic event and are probably due to the resultant pyrexia following the vaccination. Early DPT vaccinations were made by chemically treating the diphtheria, tetanus and pertussis toxins to render them non toxic yet still capable of eliciting an immune response in the vaccinated child. These early vaccines often used live adjuvant and were notorious for causing swollen arms and fever in children for a day or two after the injection. Often paracetamol or ibuprofen or other temperature lowering drugs such as aspirin or 'calpol' were given at the time of the injection in anticipation of the resultant fever. Tylenol (paracetamol) was given to the twins following their DPT vaccinations. Recent concerns about safety, have led to the developments of more acellular vaccines which are associated with fewer side effects. Although '*very common reactions*' of the modern DPT vaccine include '*being off colour and having a fever*' (National Health Service 2011b),

the modern DPT vaccinations are much less of an insult to the body (World Health Organization 2000) and as a result they may not create the accentuated vaccination/immunisation lines seen in children's teeth from twenty years ago, when Individual C and the twins received their vaccinations.

It has been known for some time that fevers (exanthematous childhood fevers, such as measles, scarlet fever, mumps and chickenpox) create linear hypoplastic defects in enamel (Beust 1937; Dean 2007; Garant 2003; Garrison 1940; Koleoso 2004; Suckling et al. 1987). However, although both exanthematous fevers and acidosis are known '*factors capable of producing enamel hypoplasia*' (Jackson 1961:213) and even though infections are also associated with an acidosis[12] which in turn reduces the degree of mineralization that is possible until the pH level returns to normal (normal pH is 7.4), the most likely cause of the hypoplasia in the case of vaccination/immunisation lines, is that of increased body temperature (fever) affecting the function of the ameloblasts (Berman 1939; Bevelander and Bernstein 1940; Garrison 1940).

The appearance of the vaccination/immunisation lines in the twins are very similar to those in Individual C's postnatal enamel. An important finding of this part of the study is that childhood inoculations often – if not always - leave a mark in enamel. This fact is extremely useful and even if the medical histories in these three case studies are not always 100% accurate, the basis of a carefully controlled study now exists; to calculate rates of enamel formation using 'labels' at known age created by vaccinations/immunisations if 'good' written histories can be released with ethical permission. This fact may well explain similar lines that have often been noted in permanent enamel (for example within the cuspal enamel of first permanent molars) which can now be explained and made use of in new ways.

[12] As mentioned before, at birth babies are often born 'blue' due to increased carbon dioxide levels and their reduced venous blood is acidotic with a low pH. The combination of poor mineralization because of the low pH and low blood calcium levels at birth are likely to underlie the formation of the neonatal line in enamel.

8.3 Further Work and Future Developments

The development of the regression formulae to calculate crown formation times, including pre- and postnatal enamel times is a new technique that could be used to assist forensic osteologists and anthropologists in estimating the age of unidentified infant remains, in order to help procure a positive identification (Boyde 1963; Skinner and Anderson 1991). This technique is limited by the fact that it can only be applied to teeth that are still forming, as once the enamel is fully formed the enamel biological clock has ‘stopped’ and this method will no longer give the age of the individual and instead will age the tooth crown formation period. So in reality this technique can be used until the age of about one year (55 weeks for the second molar tooth) as after this time crown formation is complete according to this study.

However this study has identified several other possible areas that, after further work may be of use to the forensic osteologist.

- Identification and confirmation of twins by matching normal and accentuated incremental lines.

- Identification of separated twins by matching accentuated incremental lines in prenatal enamel.

- Identification of minimum number of individuals if several deciduous teeth are present in a skeletal assemblage, again by matching the normal and accentuated incremental lines, for example two teeth that do not correspond chronologically are possibly from different individuals.

- Identification of juvenile skeletal remains by the possible matching of normal and accentuated incremental lines with potential medical histories. Although medical histories may be unreliable general trends can still be identified for example, vaccinations or operations.

- Identification of juvenile remains by maternal medical history. As demonstrated by the case study of the twins, it may be possible to identify maternal influences on the developing fetus.

- Identification of juvenile remains by identifying premature or extended delivery times from the position of the neonatal line in the enamel.

- Identification of pronounced neonatal lines that may be indicative of a caesarean delivery versus a natural birth.

- If the neonatal lines can not be identified in a particular ground section then it may be possible to use the hypoplastic decrease in the enamel formation rate to establish its probable position.

- It would also be interesting to look at teeth of young human mothers whose third molar roots are still developing, to see if there is an equivalent of a neonatal line in their teeth.

CHAPTER 9: Related Publications

The following publications are related to this study and have been included for additional information.

APPENDICES

Appendix One

Central Incisors

		Ground Section A1			Ground Section A2			Ground Section A3			Ground Section A4		
		Region			Region			Region			Region		
Aspect	µm	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal
Labial	100	33	33	34	32	32	30	30	31	30	33	31	31
Lingual	100	32	31	32	31	31	31	30	30	29	30	31	29
Labial	150	45							45				
Lingual	150							43	41	41			
Labial	200		64	62		55	54			53		56	56
Lingual	200	56	58	60								57	54
Labial	250												
Lingual	250		72									68	
Labial	300		93	87			76					81	77
Lingual	300			86									80
Labial	350											93	
Lingual	350												
Labial	400			112									99
Lingual	400												
Labial	450												109
Lingual	450												

Lateral Incisors

		Ground Section B1			Ground Section B2			Ground Section B3			Ground Section B4		
		Region			Region			Region			Region		
Aspect	µm	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal
Labial	100	36	31	30	31	35	32	34	32	30	31	35	33
Lingual	100	35	33	29	31	32	30	31	31	34	35	33	30
Labial	150	49						48					
Lingual	150				45			46	45		49		
Labial	200		56	55	58	61	56		58	53	57	63	64
Lingual	200		58	54		60	57			57		59	57
Labial	250								69		70		
Lingual	250			67		73	70					73	
Labial	300		80	80		87	80			76		91	92
Lingual	300												86
Labial	350			91									
Lingual	350												99
Labial	400		102			112	106					115	119
Lingual	400												
Labial	450						119					126	
Lingual	450												
Labial	500												146
Lingual	500												
Labial	550												
Lingual	550												
Labial	600												171
Lingual	600												

Canines

		Ground Section C1			Ground Section C2			Ground Section C3			Ground Section C4		
		Region			Region			Region			Region		
Aspect	µm	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal
Labial	100	32	33	33	31	29	28	41	33	30	31	29	29
Lingual	100	35	33	30	32	32	30	38	31	36	30	30	29
Labial	150							60					
Lingual	150										42		
Labial	200		62	63	55	52	52		62	57	54	56	52
Lingual	200		64	55	57	56	59	67	59	67		56	53
Labial	250				68								
Lingual	250				68								
Labial	300		89	88		78	79		92	84		79	78
Lingual	300					78	84	96	84	97		82	74
Labial	350		101	101									
Lingual	350												
Labial	400					104	108		121	109		100	101
Lingual	400					101	107		111	128		106	96
Labial	450												
Lingual	450						118			143		116	109
Labial	500					127	133		149	135		124	120
Lingual	500								136				
Labial	550												
Lingual	550												
Labial	600					150			175	158		147	143
Lingual	600												
Labial	650					160				169			
Lingual	650												
Labial	700								201				163
Lingual	700												
Labial	750												
Lingual	750												
Labial	800								224				
Lingual	800												
Labial	850								235				
Lingual	850												

First Molars

		Ground Section D1			Ground Section D2			Ground Section D3			Ground Section D4		
		Region			Region			Region			Region		
Aspect	µm	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal
Buccal	100	31	30	26	28	26	31	25	29	27	29	30	31
Lingual	100	29	27	25	26	30	27	26	27	26	27	27	32
Buccal	150												
Lingual	150												
Buccal	200	61	58	52	57	53	57	48	54	55	56	59	57
Lingual	200	54	52	51	48	56	52	54	51	52	53	51	56
Buccal	250				68								
Lingual	250				60			66					
Buccal	300		85	75		80	82	74	77	80	83	84	83
Lingual	300	79	77	73		80	72		77	76	75	75	79
Buccal	350										99		
Lingual	350										89		
Buccal	400		115	98		106	106		100	103		110	110
Lingual	400		102	96		102	93		103	99		99	102
Buccal	450												
Lingual	450			110									
Buccal	500		142	120		130	130		126	125		136	134
Lingual	500		125			125	114		125	121		126	123
Buccal	550		154	133			142						
Lingual	550		135										
Buccal	600					150			152	148		163	159
Lingual	600					145	134		145			150	146
Buccal	650					160							
Lingual	650					155							
Buccal	700								174			193	
Lingual	700								169			171	168
Buccal	750								183				
Lingual	750												
Buccal	800											219	
Lingual	800											194	188
Buccal	850												
Lingual	850											204	

Second Molars

		Ground Section E1			Ground Section E2			Ground Section E3			Ground Section E4		
		Region			Region			Region			Region		
Aspect	µm	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal
Buccal	100	37	31	32	34	35	41	33	35	32	38	33	36
Lingual	100	33	31	30	38	37	33	40	31	30	35	37	36
Buccal	150	55			48						55		
Lingual	150							57					
Buccal	200		63	57		67	75	62	67	59		63	61
Lingual	200	64	62	56	64	68	68		62	57	71	70	65
Buccal	250							75					
Lingual	250	79											
Buccal	300		96	85		97	104		95	85		92	87
Lingual	300		95	86		100	96		93	84		100	99
Buccal	350												
Lingual	350												
Buccal	400		128	111		128	131		123	113		120	114
Lingual	400		125	115		130	124		118	113		126	126
Buccal	450												
Lingual	450												
Buccal	500		157	139		157	159		150	140		147	138
Lingual	500		156	140		160	152		145	139		151	152
Buccal	550												
Lingual	550												
Buccal	600		185	169		184	187		176	166		171	163
Lingual	600		186			188	180		170	171		177	173
Buccal	650												175
Lingual	650												
Buccal	700		212	194		211	219		204	192		195	
Lingual	700		210			212	214			200		204	
Buccal	750												
Lingual	750					224							
Buccal	800		236			237	248		230	216		219	
Lingual	800		235				240			225		227	
Buccal	850						259						
Lingual	850									236			
Buccal	900					262			254			239	
Lingual	900		260				264					249	

Second Molars (continued)

		Ground Section E1			Ground Section E2			Ground Section E3			Ground Section E4		
		Region			Region			Region			Region		
Aspect	µm	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal	Cervical	Lateral	Occusal
Buccal	950											250	
Lingual	950												
Buccal	1000					287			281				
Lingual	1000						290						
Buccal	1050					298							
Lingual	1050												
Buccal	1100								306				
Lingual	1100						315						
Buccal	1150												
Lingual	1150												
Buccal	1200								330				
Lingual	1200						338						
Buccal	1250												
Lingual	1250												
Buccal	1300												
Lingual	1300						361						
Buccal	1350												
Lingual	1350												
Buccal	1400												
Lingual	1400						384						

Appendix Two

Central Incisors

Photomontage 1 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	23.38	23.26	543.82	142	134	149	20.2	4.65
B	10.24	23.26	238.18	66	61	70	9.4	2.16
C	5.66	23.26	131.65	39	36	43	5.6	1.29
Crown Formation Time Before Birth				142	134	149	20.2	4.65
Crown Formation Time After Birth				105	97	113	15.0	3.46
Total Crown Formation Time				**247**	**231**	**262**	**35.3**	**8.11**

Photomontage 2 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	23.01	23.26	535.21	139	132	147	19.9	4.58
B	10.29	23.26	239.35	66	62	71	9.4	2.17
C	6.72	23.26	156.31	45	42	49	6.5	1.49
Crown Formation Time Before Birth				139	132	147	19.9	4.58
Crown Formation Time After Birth				112	104	120	15.9	3.67
Total Crown Formation Time				**251**	**236**	**267**	**35.9**	**8.25**

Photomontage 3 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	23.42	23.26	544.75	142	134	149	20.3	4.66
B	8.19	23.26	190.50	54	50	58	7.7	1.77
C	4.07	23.26	94.67	30	27	33	4.3	0.99
Crown Formation Time Before Birth				142	134	149	20.3	4.66
Crown Formation Time After Birth				84	77	91	12.0	2.77
Total Crown Formation Time				**226**	**212**	**240**	**32.3**	**7.43**

Photomontage 4 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	22.57	23.26	524.98	137	130	144	19.6	4.50
B	11.73	23.26	272.84	74	70	79	10.6	2.44
C	8.18	23.26	190.27	54	50	58	7.7	1.77
Crown Formation Time Before Birth				137	130	144	19.6	4.50
Crown Formation Time After Birth				128	120	137	18.3	4.22
Total Crown Formation Time				**265**	**249**	**281**	**37.9**	**8.71**

Photomontage 5 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	24.57	23.26	571.50	148	141	156	21.2	4.88
B	6.90	23.26	160.49	47	43	50	6.6	1.53
Crown Formation Time Before Birth				148	141	156	21.2	4.88
Crown Formation Time After Birth				47	43	50	6.6	1.53
Total Crown Formation Time				**195**	**184**	**206**	**27.9**	**6.41**

Photomontage 6

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	25.86	23.26	601.50	156	148	164	22.3	5.12
B	9.15	23.26	212.83	60	55	64	8.5	1.96
C	4.81	23.26	111.88	34	31	38	4.9	1.13
Crown Formation Time Before Birth				156	148	164	22.3	5.12
Crown Formation Time After Birth				94	87	101	13.4	3.09
Total Crown Formation Time				**250**	**234**	**265**	**35.7**	**8.21**

Photomontage 7

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	23.89	23.26	555.68	145	137	152	20.6	4.75
B	11.23	23.26	261.21	72	67	76	10.2	2.35
C	5.51	23.26	128.16	39	35	42	5.5	1.27
Crown Formation Time Before Birth				145	137	152	20.6	4.75
Crown Formation Time After Birth				110	102	118	15.7	3.61
Total Crown Formation Time				**255**	**239**	**270**	**36.4**	**8.36**

Photomontage 8

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	24.87	23.26	578.48	150	142	158	21.5	4.93
B	8.96	23.26	208.41	58	54	63	8.3	1.92
C	6.27	23.26	145.84	43	39	46	6.1	1.41
D	2.31	23.26	53.73	20	17	23	2.9	0.66
Crown Formation Time Before Birth				150	142	158	21.5	4.93
Crown Formation Time After Birth				121	111	132	17.3	3.99
Total Crown Formation Time				**272**	**253**	**290**	**38.8**	**8.92**

Photomontage 9 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	22.42	23.26	521.49	136	129	143	19.4	4.47
B	14.82	23.26	344.71	92	87	98	13.2	3.03
C	4.50	23.26	104.67	33	30	36	4.7	1.07
Crown Formation Time Before Birth				136	129	143	19.4	4.47
Crown Formation Time After Birth				125	116	134	17.8	4.10
Total Crown Formation Time				**261**	**245**	**277**	**37.3**	**8.57**

Photomontage 10 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	24.45	23.26	568.71	148	140	156	21.1	4.85
B	5.07	23.26	117.93	36	33	39	5.1	1.18
Crown Formation Time Before Birth				148	140	156	21.1	4.85
Crown Formation Time After Birth				36	33	39	5.1	1.18
Total Crown Formation Time				**184**	**173**	**195**	**26.2**	**6.04**

Mean
Enamel Formation Time (days) = 0.248 x Enamel Thickness (µm) + 6.731
Lower 95% Confidence Limit
Enamel Formation Time (days) = 0.238 x Enamel Thickness (µm) + 4.678
Upper 95% Confidence Limit
Enamel Formation Time (days) = 0.258 x Enamel Thickness (µm) + 8.784

Lateral Incisors

Photomontage 1

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	20.98	22.55	473.10	131	126	136	18.7	4.29
B	8.49	22.55	191.45	56	53	59	8.0	1.84
C	13.79	22.55	310.96	88	84	92	12.5	2.88
Crown Formation Time Before Birth				131	126	136	18.7	4.29
Crown Formation Time After Birth				144	137	151	20.5	4.73
Total Crown Formation Time				**275**	**262**	**287**	**39.2**	**9.02**

Photomontage 2 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	22.94	22.55	517.30	142	137	148	20.3	4.68
B	12.27	22.55	276.69	79	75	82	11.2	2.58
Crown Formation Time Before Birth				142	137	148	20.3	4.68
Crown Formation Time After Birth				79	75	82	11.2	2.58
Total Crown Formation Time				**221**	**212**	**230**	**31.6**	**7.26**

Photomontage 3

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	22.98	22.55	518.20	143	137	148	20.4	4.69
B	11.90	22.55	268.35	76	73	80	10.9	2.51
C	10.48	22.55	236.32	68	65	71	9.7	2.23
Crown Formation Time Before Birth				143	137	148	20.4	4.69
Crown Formation Time After Birth				144	137	152	20.6	4.74
Total Crown Formation Time				**287**	**275**	**300**	**41.0**	**9.43**

Photomontage 4

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	21.92	22.55	494.30	136	131	142	19.5	4.48
B	17.19	22.55	387.63	108	104	113	15.4	3.55
Crown Formation Time Before Birth				136	131	142	19.5	4.48
Crown Formation Time After Birth				108	104	113	15.4	3.55
Total Crown Formation Time				**244**	**235**	**254**	**34.9**	**8.03**

Photomontage 5

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	20.65	22.55	465.66	129	124	134	18.4	4.23
B	8.44	22.55	190.32	56	53	59	8.0	1.83
C	4.50	22.55	101.48	32	30	35	4.6	1.06
Crown Formation Time Before Birth				129	124	134	18.4	4.23
Crown Formation Time After Birth				88	82	94	12.6	2.89
Total Crown Formation Time				**217**	**206**	**227**	**31.0**	**7.12**

Photomontage 6

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	21.98	22.55	495.65	137	131	142	19.5	4.49
B	6.01	22.55	135.53	41	39	44	5.9	1.36
C	13.84	22.55	312.09	88	84	92	12.6	2.89
Crown Formation Time Before Birth				137	131	142	19.5	4.49
Crown Formation Time After Birth				129	123	136	18.5	4.25
Total Crown Formation Time				**266**	**254**	**278**	**38.0**	**8.74**

Photomontage 7

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	21.28	22.55	479.86	133	127	138	18.9	4.35
B	7.27	22.55	163.94	49	46	52	7.0	1.60
C	3.30	22.55	74.42	25	23	27	3.6	0.82
Crown Formation Time Before Birth				133	127	138	18.9	4.35
Crown Formation Time After Birth				74	69	79	10.5	2.43
Total Crown Formation Time				**206**	**196**	**217**	**29.5**	**6.78**

Photomontage 8

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	22.30	22.55	502.87	139	133	144	19.8	4.55
B	11.02	22.55	248.50	71	68	75	10.2	2.34
C	5.56	22.55	125.38	39	36	41	5.5	1.27
Crown Formation Time Before Birth				139	133	144	19.8	4.55
Crown Formation Time After Birth				110	104	116	15.7	3.61
Total Crown Formation Time				**248**	**237**	**260**	**35.5**	**8.16**

Photomontage 9

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	22.57	22.55	508.95	140	135	146	20.0	4.61
B	15.83	22.55	356.97	100	96	104	14.3	3.28
C	4.81	22.55	108.47	34	32	37	4.9	1.12
Crown Formation Time Before Birth				140	135	146	20.0	4.61
Crown Formation Time After Birth				134	127	141	19.1	4.40
Total Crown Formation Time				**274**	**262**	**286**	**39.2**	**9.01**

Photomontage 10

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	21.88	22.55	493.39	136	131	141	19.4	4.47
B	9.99	22.55	225.27	65	62	68	9.3	2.14
C	7.56	22.55	170.48	51	48	54	7.2	1.66
Crown Formation Time Before Birth				136	131	141	19.4	4.47
Crown Formation Time After Birth				116	109	122	16.5	3.80
Total Crown Formation Time				**252**	**240**	**263**	**35.9**	**8.27**

Mean
Enamel Formation Time (days) = 0.265 x Enamel Thickness (µm) + 5.342
Lower 95% Confidence Limit
Enamel Formation Time (days) = 0.258 x Enamel Thickness (µm) + 3.535
Upper 95% Confidence Limit
Enamel Formation Time (days) = 0.272 x Enamel Thickness (µm) + 7.148

Canines

Photomontage 1

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	22.38	22.60	505.79	135	128	142	19.3	4.44
B	13.89	22.60	313.91	87	81	92	12.4	2.84
C	15.74	22.60	355.72	97	91	103	13.9	3.19
D	12.94	22.60	292.44	81	75	86	11.6	2.66
Crown Formation Time Before Birth				135	128	142	19.3	4.44
Crown Formation Time After Birth				265	247	281	37.8	8.70
Total Crown Formation Time				**400**	**375**	**423**	**57.1**	**13.13**

Photomontage 2

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	26.09	22.60	589.63	156	148	164	22.3	5.13
B	18.83	22.60	425.56	115	108	121	16.4	3.77
C	10.76	22.60	243.18	69	63	74	9.8	2.25
D	7.37	22.60	166.56	49	45	54	7.0	1.62
Crown Formation Time Before Birth				156	148	164	22.3	5.13
Crown Formation Time After Birth				233	216	248	33.2	7.64
Total Crown Formation Time				**389**	**364**	**412**	**55.6**	**12.78**

Photomontage 3 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	19.95	22.60	450.87	121	114	128	17.3	3.98
B	17.01	22.60	384.43	104	98	110	14.9	3.43
C	14.92	22.60	337.19	92	86	98	13.2	3.04
D	7.57	22.60	171.08	50	46	55	7.2	1.66
E	4.95	22.60	111.87	35	31	39	5.1	1.16
Crown Formation Time Before Birth				121	114	128	17.3	3.98
Crown Formation Time After Birth				283	262	303	40.4	9.28
Total Crown Formation Time				**404**	**376**	**430**	**57.7**	**13.27**

Photomontage 4

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	16.13	22.60	364.54	99	93	105	14.2	3.26
B	12.42	22.60	280.69	78	73	83	11.2	2.57
C	12.31	22.60	278.21	77	72	83	11.1	2.55
D	10.22	22.60	230.97	66	60	70	9.4	2.15
E	6.34	22.60	143.28	43	39	47	6.2	1.42
F	3.57	22.60	80.68	28	24	31	3.9	0.90
Crown Formation Time Before Birth				99	93	105	14.2	3.26
Crown Formation Time After Birth				292	268	315	41.7	9.59
Total Crown Formation Time				**391**	**361**	**420**	**55.9**	**12.86**

Photomontage 5 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	23.75	22.60	536.75	143	135	150	20.4	4.69
B	7.54	22.60	170.40	50	46	55	7.2	1.65
C	9.84	22.60	222.38	63	58	68	9.1	2.08
D	9.51	22.60	214.93	61	57	66	8.8	2.02
E	6.73	22.60	152.10	46	41	50	6.5	1.50
F	5.50	22.60	124.30	39	34	43	5.5	1.27
G	5.29	22.60	119.55	37	33	41	5.3	1.23
Crown Formation Time Before Birth				143	135	150	20.4	4.69
Crown Formation Time After Birth				297	270	322	42.4	9.74
Total Crown Formation Time				**439**	**405**	**473**	**62.8**	**14.44**

Photomontage 6

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	26.77	22.60	605.00	160	152	168	22.9	5.26
B	31.57	22.60	713.48	188	178	196	26.8	6.16
C	14.53	22.60	328.38	90	84	96	12.9	2.96
D	12.28	22.60	277.53	77	72	83	11.0	2.54
Crown Formation Time Before Birth				160	152	168	22.9	5.26
Crown Formation Time After Birth				355	334	375	50.7	11.67
Total Crown Formation Time				**515**	**486**	**543**	**73.6**	**16.93**

Photomontage 7

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	18.48	22.60	417.65	113	106	119	16.1	3.71
B	19.42	22.60	438.89	118	111	125	16.9	3.88
C	17.64	22.60	398.66	108	101	114	15.4	3.55
D	11.62	22.60	262.61	74	68	79	10.5	2.42
Crown Formation Time Before Birth				113	106	119	16.1	3.71
Crown Formation Time After Birth				300	281	317	42.8	9.85
Total Crown Formation Time				**412**	**387**	**437**	**58.9**	**13.55**

Photomontage 8 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	24.50	22.60	553.70	147	139	155	21.0	4.84
B	17.13	22.60	387.14	105	99	111	15.0	3.45
C	11.88	22.60	268.49	75	70	80	10.7	2.47
D	7.40	22.60	167.24	49	45	54	7.1	1.62
E	5.27	22.60	119.10	37	33	41	5.3	1.22
Crown Formation Time Before Birth				147	139	155	21.0	4.84
Crown Formation Time After Birth				267	246	286	38.1	8.76
Total Crown Formation Time				**414**	**386**	**441**	**59.1**	**13.60**

Photomontage 9 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	16.58	22.60	374.71	102	96	108	14.6	3.35
B	17.25	22.60	389.85	106	99	112	15.1	3.47
C	17.37	22.60	392.56	106	100	113	15.2	3.50
D	13.47	22.60	304.42	84	78	90	12.0	2.76
E	7.02	22.60	158.65	47	43	51	6.7	1.55
F	5.02	22.60	113.45	36	32	40	5.1	1.18
Crown Formation Time Before Birth				102	96	108	14.6	3.35
Crown Formation Time After Birth				379	352	405	54.2	12.46
Total Crown Formation Time				**481**	**448**	**513**	**68.7**	**15.81**

Photomontage 10

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	17.67	22.60	399.34	108	102	114	15.4	3.55
B	19.97	22.60	451.32	121	114	128	17.3	3.98
C	13.90	22.60	314.14	87	81	92	12.4	2.84
D	11.83	22.60	267.36	75	69	80	10.7	2.46
E	9.98	22.60	225.55	64	59	69	9.2	2.11
Crown Formation Time Before Birth				108	102	114	15.4	3.55
Crown Formation Time After Birth				347	324	369	49.5	11.39
Total Crown Formation Time				**455**	**425**	**483**	**65.0**	**14.95**

Mean
Enamel Formation Time (days) = 0.253 x Enamel Thickness (µm) + 7.106
Lower 95% Confidence Limit
Enamel Formation Time (days) = 0.244 x Enamel Thickness (µm) + 4.123
Upper 95% Confidence Limit
Enamel Formation Time (days) = 0.261 x Enamel Thickness (µm) + 10.089

First Molars

Photomontage 1

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	16.85	22.56	380.14	100	95	104	14.3	3.28
B	21.92	22.56	494.52	129	124	134	18.4	4.23
C	12.11	22.56	273.20	73	69	77	10.4	2.39
D	9.84	22.56	221.99	60	56	63	8.5	1.96
Crown Formation Time Before Birth				100	95	104	14.3	3.28
Crown Formation Time After Birth				261	248	274	37.3	8.58
Total Crown Formation Time				**361**	**344**	**379**	**51.6**	**11.86**

Photomontage 2 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	27.91	22.56	629.65	163	157	169	23.3	5.36
B	12.20	22.56	275.23	73	69	77	10.5	2.40
C	9.49	22.56	214.09	58	54	61	8.2	1.89
D	7.16	22.56	161.53	44	41	48	6.3	1.46
E	5.43	22.56	122.50	34	31	37	4.9	1.13
Crown Formation Time Before Birth				163	157	169	23.3	5.36
Crown Formation Time After Birth				210	196	224	29.9	6.89
Total Crown Formation Time				**373**	**353**	**393**	**53.3**	**12.25**

Photomontage 3 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	26.93	22.56	607.54	158	152	164	22.5	5.18
B	14.33	22.56	323.28	85	81	90	12.2	2.81
C	7.79	22.56	175.74	48	45	51	6.8	1.57
D	7.22	22.56	162.88	45	41	48	6.4	1.47
Crown Formation Time Before Birth				158	152	164	22.5	5.18
Crown Formation Time After Birth				178	167	189	25.4	5.85
Total Crown Formation Time				**336**	**319**	**353**	**47.9**	**11.03**

Photomontage 4

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	24.41	22.56	550.69	143	138	149	20.5	4.70
B	17.11	22.56	386.00	101	97	106	14.5	3.33
C	13.20	22.56	297.79	79	75	83	11.3	2.59
D	9.98	22.56	225.15	60	57	64	8.6	1.99
Crown Formation Time Before Birth				143	138	149	20.5	4.70
Crown Formation Time After Birth				241	228	253	34.4	7.91
Total Crown Formation Time				**384**	**366**	**402**	**54.8**	**12.61**

Photomontage 5

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	26.03	22.56	587.24	152	147	158	21.8	5.01
B	10.46	22.56	235.98	63	59	67	9.0	2.08
C	8.69	22.56	196.05	53	50	57	7.6	1.74
Crown Formation Time Before Birth				152	147	158	21.8	5.01
Crown Formation Time After Birth				116	109	124	16.6	3.82
Total Crown Formation Time				**269**	**256**	**282**	**38.4**	**8.83**

Photomontage 6 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	20.44	22.56	461.13	120	115	126	17.2	3.96
B	13.66	22.56	308.17	82	77	86	11.7	2.68
C	9.64	22.56	217.48	59	55	62	8.4	1.92
D	8.71	22.56	196.50	53	50	57	7.6	1.75
	Crown Formation Time Before Birth			120	115	126	17.2	3.96
	Crown Formation Time After Birth			193	182	205	27.6	6.35
	Total Crown Formation Time			**314**	**297**	**330**	**44.8**	**10.31**

Photomontage 7 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	25.19	22.56	568.29	148	142	153	21.1	4.85
B	12.82	22.56	289.22	77	73	81	11.0	2.52
C	8.64	22.56	194.92	53	49	56	7.5	1.73
	Crown Formation Time Before Birth			148	142	153	21.1	4.85
	Crown Formation Time After Birth			130	122	137	18.5	4.26
	Total Crown Formation Time			**277**	**264**	**291**	**39.6**	**9.11**

Photomontage 8 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	19.05	22.56	429.77	112	108	117	16.1	3.69
B	7.15	22.56	161.30	44	41	48	6.3	1.45
C	8.99	22.56	202.81	55	51	58	7.8	1.80
D	8.33	22.56	187.92	51	48	54	7.3	1.68
E	6.07	22.56	136.94	38	35	41	5.4	1.25
	Crown Formation Time Before Birth			112	108	117	16.1	3.69
	Crown Formation Time After Birth			188	175	202	26.9	6.18
	Total Crown Formation Time			**301**	**282**	**319**	**42.9**	**9.88**

Photomontage 9 - NNL Reconstructed

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	25.81	22.56	582.27	151	145	157	21.6	4.97
B	17.85	22.56	402.70	106	101	110	15.1	3.47
C	17.81	22.56	401.79	105	101	110	15.0	3.46
	Crown Formation Time Before Birth			151	145	157	21.6	4.97
	Crown Formation Time After Birth			211	201	220	30.1	6.93
	Total Crown Formation Time			**362**	**347**	**377**	**51.7**	**11.90**

Photomontage 10

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	26.33	22.56	594.00	154	148	160	22.0	5.07
B	12.40	22.56	279.74	74	70	78	10.6	2.44
C	9.17	22.56	206.88	56	52	59	8.0	1.83
	Crown Formation Time Before Birth			154	148	160	22.0	5.07
	Crown Formation Time After Birth			130	123	138	18.6	4.28
	Total Crown Formation Time			**284**	**271**	**298**	**40.6**	**9.34**

Mean
Enamel Formation Time (days) = 0.254 x Enamel Thickness (µm) + 3.291
Lower 95% Confidence Limit
Enamel Formation Time (days) = 0.248 x Enamel Thickness (µm) + 0.951
Upper 95% Confidence Limit
Enamel Formation Time (days) = 0.260 x Enamel Thickness (µm) + 5.631

Second Molars

Photomontage 1

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	18.81	22.49	423.04	127	123	132	18.2	4.19
B	17.18	22.49	386.38	117	113	121	16.8	3.86
C	27.40	22.49	616.23	180	175	185	25.8	5.93
D	4.97	22.49	111.78	42	39	45	6.0	1.39
E	6.89	22.49	154.96	54	51	57	7.7	1.77
Crown Formation Time Before Birth				127	123	132	18.2	4.19
Crown Formation Time After Birth				394	378	408	56.3	12.94
Total Crown Formation Time				**521**	**501**	**540**	**74.5**	**17.13**

Photomontage 2

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	14.88	22.49	334.65	103	99	107	14.7	3.39
B	11.61	22.49	261.11	83	79	86	11.9	2.73
C	11.99	22.49	269.66	85	82	89	12.2	2.81
D	11.38	22.49	255.94	82	78	85	11.7	2.68
E	8.77	22.49	197.24	66	62	69	9.4	2.15
F	5.20	22.49	116.95	44	41	46	6.2	1.43
G	7.29	22.49	163.95	56	53	59	8.1	1.86
Crown Formation Time Before Birth				103	99	107	14.7	3.39
Crown Formation Time After Birth				416	395	435	59.4	13.66
Total Crown Formation Time				**519**	**495**	**542**	**74.2**	**17.05**

Photomontage 3

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	18.32	22.49	412.02	124	120	128	17.8	4.09
B	32.90	22.49	739.92	214	208	220	30.6	7.04
C	20.25	22.49	455.42	136	132	141	19.5	4.48
D	6.79	22.49	152.71	53	50	56	7.6	1.75
Crown Formation Time Before Birth				124	120	128	17.8	4.09
Crown Formation Time After Birth				404	390	416	57.7	13.27
Total Crown Formation Time				**528**	**510**	**545**	**75.5**	**17.36**

Photomontage 4

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	16.56	22.49	372.43	114	109	117	16.2	3.73
B	15.22	22.49	342.30	105	101	109	15.0	3.46
C	16.41	22.49	369.06	113	108	117	16.1	3.70
D	14.42	22.49	324.31	100	96	104	14.3	3.30
E	11.59	22.49	260.66	83	79	86	11.9	2.73
Crown Formation Time Before Birth				114	109	117	16.2	3.73
Crown Formation Time After Birth				401	385	416	57.3	13.19
Total Crown Formation Time				**515**	**495**	**533**	**73.6**	**16.92**

Photomontage 5

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	Confidence Limits 95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	15.09	22.49	339.37	105	100	108	14.9	3.43
B	24.18	22.49	543.81	161	155	165	22.9	5.27
C	15.32	22.49	344.55	106	102	110	15.1	3.48
D	10.23	22.49	230.07	75	71	78	10.7	2.45
Crown Formation Time Before Birth				105	100	108	14.9	3.43
Crown Formation Time After Birth				341	328	353	48.7	11.21
Total Crown Formation Time				**446**	**429**	**461**	**63.7**	**14.64**

Photomontage 6

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	16.99	22.49	382.11	116	112	120	16.6	3.82
B	43.12	22.49	969.77	277	270	283	39.6	9.11
C	14.06	22.49	316.21	98	94	102	14.0	3.23
D	4.91	22.49	110.43	42	39	45	6.0	1.37
Crown Formation Time Before Birth				116	112	120	16.6	3.82
Crown Formation Time After Birth				417	403	430	59.6	13.71
Total Crown Formation Time				**534**	**515**	**550**	**76.2**	**17.53**

Photomontage 7

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	14.85	22.49	333.98	103	99	107	14.7	3.39
B	33.76	22.49	759.26	220	213	225	31.4	7.21
C	9.35	22.49	210.28	69	66	72	9.9	2.27
D	7.66	22.49	172.27	59	56	62	8.4	1.93
Crown Formation Time Before Birth				103	99	107	14.7	3.39
Crown Formation Time After Birth				348	335	359	49.6	11.42
Total Crown Formation Time				**451**	**434**	**466**	**64.4**	**14.80**

Photomontage 8

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	16.67	22.49	374.91	114	110	118	16.3	3.75
B	25.01	22.49	562.47	166	160	170	23.7	5.44
C	11.31	22.49	254.36	81	78	85	11.6	2.67
D	11.08	22.49	249.19	80	76	83	11.4	2.62
E	10.21	22.49	229.62	74	71	78	10.6	2.45
Crown Formation Time Before Birth				114	110	118	16.3	3.75
Crown Formation Time After Birth				401	385	416	57.3	13.18
Total Crown Formation Time				**515**	**495**	**534**	**73.6**	**16.94**

Photomontage 9

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	19.20	22.49	431.81	130	125	134	18.6	4.27
B	21.04	22.49	473.19	141	136	145	20.2	4.64
C	16.16	22.49	363.44	111	107	115	15.9	3.65
D	13.93	22.49	313.29	97	93	101	13.9	3.20
E	5.43	22.49	122.12	45	42	48	6.4	1.48
Crown Formation Time Before Birth				130	125	134	18.6	4.27
Crown Formation Time After Birth				395	379	409	56.4	12.97
Total Crown Formation Time				**525**	**504**	**543**	**74.9**	**17.24**

Photomontage 10

Measurement	Prism Length (mm)	Magnification Factor (mm)	Prism Length (µm)	Mean (days)	Confidence Limits 95% Lower (days)	95% Upper (days)	Weeks (mean days/7)	Months (mean days/30.44)
A	20.75	22.49	466.67	139	135	144	19.9	4.58
B	12.26	22.49	275.73	87	83	91	12.4	2.86
C	13.27	22.49	298.44	93	89	97	13.3	3.07
D	8.83	22.49	198.59	66	63	69	9.4	2.17
E	11.42	22.49	256.84	82	78	85	11.7	2.69
F	4.96	22.49	111.55	42	39	45	6.0	1.38
Crown Formation Time Before Birth				139	135	144	19.9	4.58
Crown Formation Time After Birth				370	353	387	52.9	12.17
Total Crown Formation Time				**510**	**488**	**530**	**72.8**	**16.75**

Mean
Enamel Formation Time (days) = 0.274 x Enamel Thickness (µm) + 11.548
Lower 95% Confidence Limit
Enamel Formation Time (days) = 0.269 x Enamel Thickness (µm) + 9.194
Upper 95% Confidence Limit
Enamel Formation Time (days) = 0.278 x Enamel Thickness (µm) + 13.902

Appendix Three

Central Incisors

| | | Cervical Region per 100µm Zone | | | | | | Lateral Region per 100µm Zone | | | | | | | | | | | | | | | | | | | | | | | | Occlusal Region per 100µm Zone | | | | | | | | | | | | | | | | | | |

Lateral Incisors

| | | Cervical Region per 100µm Zone | | | | | | Lateral Region per 100µm Zone | | | | | | | | | | | | | | | | | | | | | | | | Occlusal Region per 100µm Zone | | | | | | | | | | | | | | | | | | |

Canines

| | | Cervical Region per 100µm Zone | | | | | | Lateral Region per 100µm Zone | | | | | | | | | | | | | | | | | | | | | | | | | | Occlusal Region per 100µm Zone | | | | | | | | | | | | | | | | | | |

First Molars

| | | Cervical Region per 100µm Zone | | | | | | Lateral Region per 100µm Zone | | | | | |

Second Molars

| | | Cervical Region per 100µm Zone | | | | | | Lateral Region per 100µm Zone | | | | | |

Appendix Four

Weighted averages calculated from the means of the raw data in **Appendix Three**

	Regional Cervical Mean	Regional Lateral Mean	Regional Occlusal Mean	Tooth Type Weighted Mean
Central Incisor	2.86	2.81	3.14	**2.96**
Lateral Incisor	2.80	3.03	3.21	**3.05**
Canine	3.05	3.21	3.29	**3.21**
First Molar	3.44	3.54	3.71	**3.58**
Second Molar	2.66	3.22	3.13	**3.11**
Regional Weighted Mean	**3.01**	**3.24**	**3.31**	**3.23**

Number of Data Points

	Cervical	Lateral	Occlusal	Total
Central Incisor	110	200	230	**540**
Lateral Incisor	160	260	300	**720**
Canine	170	420	402	**992**
First Molar	250	580	480	**1310**
Second Molar	180	730	650	**1560**
Total	**870**	**2190**	**2062**	**5122**

$$\frac{\text{(Average A x No of Data Points In A) + (Average B x No of Data Points In B) + (Average C x No of Data Points In C)...}}{\text{Total Number of Data Points}}$$

Tooth Type Weighted Calculations

(2.86 x 110) + (2.81 x 200) + (3.14 x 230) / 540
(2.80 x 160) + (3.03 x 260) + (3.21 x 300) / 720
(3.05 x 170) + (3.21 x 420) + (3.29 x 402) / 992
(3.44 x 250) + (3.54 x 580) + (3.71 x 480) / 1310
(2.66 x 180) + (3.22 x 730) + (3.13 x 650) / 1560

Regional Weighted Calculations

(2.86 x 110) + (2.80 x 160) + (3.05 x 170) + (3.44 x 250) + (2.66 x 180) / 870
(2.81 x 200) + (3.03 x 260) + (3.21 x 420) + (3.54 x 580) + (3.22 x 730) / 2190
(3.14 x 230) + (3.21 x 300) + (3.29 x 402) + (3.71 x 480) + (3.13 x 650) / 2062

Total Weighted Average Calculation

(3.01 x 870) + (3.24 x 2190) + (3.31 x 2062) / 5122

Weighted averages calculated from the means of the raw data in **Appendix Three**

For EDJ by Tooth and Region

	Regional Cervical Mean	Regional Lateral Mean	Regional Occlusal Mean	Tooth Type Weighted Mean
Central Incisor	2.74	2.68	2.83	**2.75**
Lateral Incisor	2.67	2.81	2.86	**2.78**
Canine	2.84	2.92	2.94	**2.90**
First Molar	3.25	3.32	3.37	**3.31**
Second Molar	2.49	2.55	2.48	**2.51**
Regional Weighted Mean	**2.80**	**2.86**	**2.90**	**2.85**

For Enamel Surface by Tooth and Region

	Regional Cervical Mean	Regional Lateral Mean	Regional Occlusal Mean	Tooth Type Weighted Mean
Central Incisor	2.91	2.87	3.45	**3.08**
Lateral Incisor	2.90	3.14	3.60	**3.21**
Canine	3.09	3.55	3.61	**3.42**
First Molar	3.60	3.99	3.78	**3.79**
Second Molar	2.79	4.00	3.74	**3.51**
Regional Weighted Mean	**3.06**	**3.51**	**3.64**	**3.40**

Number of Data Points

	Cervical	Lateral	Occlusal	Total
Central Incisor	80	80	80	**240**
Lateral Incisor	80	80	80	**240**
Canine	80	80	80	**240**
First Molar	80	80	80	**240**
Second Molar	80	80	80	**240**
Total	**400**	**400**	**400**	**1200**

Tooth Type Weighted Calculations EDJ
(2.74 x 80) + (2.68 x 80) + (3.83 x 80) / 240
(2.67 x 80) + (2.81 x 80) + (2.86 x 80) / 240
(2.84 x 80) + (2.92 x 80) + (3.32 x 80) / 240
(3.25 x 80) + (3.32 x 80) + (3.37 x 80) / 240
(2.49 x 80) + (2.55 x 80) + (2.48 x 80) / 240

Regional Weighted Calculations
(2.74 x 80) + (2.67 x 80) + (2.84 x 80) + (3.25 x 80) + (2.49 x 80) / 400
(2.68 x 80) + (2.81 x 80) + (2.92 x 80) + (3.32 x 80) + (2.55 x 80) / 400
(2.83 x 80) + (2.86 x 80) + (2.94 x 80) + (3.37 x 80) + (2.48 x 80) / 400

Total Weighted Average Calculation
(2.80 x 400) + (2.86 x 400) + (2.90 x 400) / 1200

Tooth Type Weighted Calculations Enamel Surface
(2.91 x 80) + (2.87 x 80) + (3.45 x 80) / 240
(2.90 x 80) + (3.14 x 80) + (3.60 x 80) / 240
(3.09 x 80) + (3.55 x 80) + (3.61 x 80) / 240
(3.60 x 80) + (3.99 x 80) + (3.78 x 80) / 240
(2.79 x 80) + (4.00 x 80) + (3.74 x 80) / 240

Regional Weighted Calculations
(2.91 x 80) + (2.90 x 80) + (3.09 x 80) + (3.60 x 80) + (2.79 x 80) / 400
(2.87 x 80) + (3.14 x 80) + (3.55 x 80) + (3.99 x 80) + (4.00 x 80) / 400
(3.45 x 80) + (3.60 x 80) + (3.61 x 80) + (3.78 x 80) + (3.74 x 80) / 400

Total Weighted Average Calculation
(3.06 x 400) + (3.51 x 400) + (3.64 x 400) / 1200

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